REMARKS

A check for $510 for a three-month extension of time accompanies this response. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 1-3, 10-13, 19, 20, 34-36, 40-46, 48-55, 108, 109, 113-116, 118-120 and 122-126 are pending. Claims 11-13, 34, 43-46, 48-55, 115, 116, 118-120 and 122-126 are withdrawn from consideration as directed to non-elected subject matter but are retained for possible rejoinder. Claim 5 is canceled herein without prejudice or disclaimer. Claims 1, 12, 13 and 19 are amended herein.

Basis for the amendment is found throughout the specification (*e.g.*, see page 50, lines 16-17 and 27-32). Claims 12 and 13 are amended for clarity. Basis for the amendment is found throughout the specification (*e.g.*, see page 25, lines 22-27). Claim 19 is amended for clarity. Basis for the amendment is found throughout the specification (*e.g.*, see page 10, lines 3-13). No new matter is added. Claim 1 is amended for clarity to render it clear that the claim is directed an isolated single chain MTSP protease domain polypeptide or catalytically active portion thereof. Claim 1 incorporates the limitations of claim 5, which is cancelled herein without prejudice or disclaimer. Hence claim 1 does not read on any full-length MTSP, but reads on the isolated single chain protease domains. No new matter is added.

The instant application shows that isolated protease domains of this family of proteases exhibit activity as a single chain that contains only the protease domain. The understanding in the art, as discussed extensively in previous responses, was that activation cleavage was required to produce even a protease domain with protease activity and that such activity required a two chain structure that includes the protease domain **plus** additional amino acids bonded thereto as a second chain. Such understanding is evidenced in the art of record (see, *e.g.*, Takeuchi *et al.*), which teaches that activation cleavage is required for activity. No art of record discloses *an isolated single chain protease domain* of any MTSP as claimed. Further, the instant application, as discussed below and in previous responses of record provides working examples demonstrating that such isolated protease domains possess protease activity as a single chain. The instant application provides at least 18 examples of such proteinase. Hence, claim 1, which is generic to and encompasses the elected species should be allowable. Applicant has made a generic discovery – the activity of isolated single-

chain protease domains – and should be entitled to a claim commensurate in scope with such discovery.

I. OBJECTION TO CLAIMS 11-13 AND 34 AS ALLEGEDLY DIRECTED TO NON-ELECTED SUBJECT MATTER

Claims 11-13 and 34 are objected to for allegedly being drawn to non-elected subject matter. Claims 11-13 and 34, which are withdrawn from consideration, are retained pending a determination of the allowability of claim 1, which is a linking claim linking the elected subject matter with the subject matter of the withdrawn claims. If claim 1 is allowed, then the non-elected subject matter in these claims, which are within the scope of claim 1, will be allowable.

II. THE REJECTION OF CLAIMS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The bases set forth by the Examiner are discussed in turn below. This rejection is respectfully traversed.

Relevant Law

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). A claim is not indefinite when one skilled in the art would understand the language in the claims when read in light of the specification.

35 U.S.C. § 112, second paragraph requires only reasonable precision in delineating the bounds of the claimed invention. Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

1) "Substantially purified"

Claims 1-3, 5 and 11-13 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner urges that the metes and bounds of the recitation "substantially purified single-chain polypeptide" in claim 1 is not clear because the Examiner alleges that the specification does not provide a clear definition for the phrase "substantially purified."

Applicant respectfully disagrees. First, the claim recites "isolated, substantially purified." Second, the specification defines what is meant by a compound such as a polypeptide being substantially pure. For example, page 46, lines 4-15 recites:

> As used herein, substantially pure means sufficiently homogeneous to appear free of readily detectable impurities as determined by standard methods of analysis, such as thin layer chromatography (TLC), gel electrophoresis and high performance liquid chromatography (HPLC), used by those of skill in the art to assess such purity, or sufficiently pure such that further purification would not detectably alter the physical and chemical properties, such as enzymatic and biological activities, of the substance. Methods for purification of the compounds to produce substantially chemically pure compounds are known to those of skill in the art. A substantially chemically pure compound may, however, be a mixture of stereoisomers or isomers. In such instances, further purification might increase the specific activity of the compound.

Thus, applicant respectfully submits that, when read in light of the specification, the skilled artisan would understand the meaning of the recitation "substantially purified" as recited in the claims and would be able to determine the metes and bounds of claims 1-3, 5, 11, 12 and 13. Applicant respectfully submits that, because the skilled artisan would understand the language and scope of the claims when read in light of the specification, the claims are not indefinite.

2) "From MTSP"

Claims 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner alleges that it is not clear as to how the skilled artisan would be able to identify a given amino acid sequence as being "from MTSP" or not being "from MTSP" in the recitation "the MTSP protease domain or catalytically active fragment thereof is the only portion of the single-chain polypeptide from the MTSP."

Without addressing the propriety of the rejection, the rejection is moot in light of the amendment of claim 1 herein. As amended, none of the claims include the recitation "the MTSP protease domain or catalytically active fragment thereof is the only portion of the single-chain polypeptide from the MTSP."

3) "Sequence of amino acids set forth as ..."

Claims 12, 13, 113 and 114 and are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner alleges that the metes and bounds of the claims are not clear because it is not clear to the Examiner whether the recited amino acid sequence has a sequence of amino acid residues set forth as amino acids 615-855 of SEQ ID

NO:2 or is a representative member of a genus. The Examiner suggests amending the claims to recite "protease domain comprises amino acids 615-855 of SEQ ID NO:2" in order to "clearly indicate that protease domain has the amino acids 615-855 of SEQ ID NO:2."

Without addressing the propriety of the rejection, in order to advance prosecution of the application to allowance, claims 12 and 13 are amended herein as suggested by the Examiner, except to be consistent with the amendment of claim 1 herein. Applicant respectfully submits that the rejection is obviated by the amendment of claims 12 and 13 herein. Reconsideration and withdrawal of the rejection are respectfully requested.

4) "Free Cys"

Claims 19 and 20 and are rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner alleges that the metes and bounds of the claims are not clear because the Examiner alleges that the specification does not provide a clear definition for the phrase "free Cys."

Applicant respectfully disagrees. The specification defines what is meant by a "free Cys" as used in the claims. For example, page 10, lines 3-13 recites:

> Also provided are muteins of the single chain protease domains and MTSPs, particularly muteins in which the Cys residue in the protease domain that is free (*i.e.*, does not form disulfide linkages with any other Cys residue in the protein) is substituted with another amino acid substitution, preferably with a conservative amino acid substitution or a substitution that does not eliminate the activity, and muteins in which a glycosylation site(s) is eliminated. Muteins in which other conservative amino acid substitutions in which catalytic activity is retained are also contemplated (see, *e.g.*, Table 1, for exemplary amino acid substitutions). See, also, Figure 4, which identifies the free Cys residues in MTSP3, MTSP4 and MTSP6.

Thus, applicant respectfully submits that, when read in light of the specification, the skilled artisan would understand the meaning of the recitation "free Cys" as recited in the claims to refer to a Cys residue that does not form disulfide linkages with any other Cys residue in the protein and thus would be able to determine the metes and bounds of claims 19 and 20. Applicant respectfully submits that, because the skilled artisan would understand the language and scope of the claims when read in light of the specification, the claims are not indefinite.

5) "Exhibits proteolytic activity"

Claim 19 is rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite, because the Examiner alleges that meaning of the recitation "exhibits proteolytic activity" is not clear and thus the metes and bounds of the claims cannot be determined.

The rejection is obviated by the amendment of claim 19 herein. As amended, claim 19 recites that the polypeptide resulting from replacing a free Cys in the protease domain with another amino acid retains serine protease activity. Applicant respectfully submits that the skilled artisan would understand the language and scope of the claims when read in light of the specification, and hence claim 19 is not indefinite.

III. REJECTION OF CLAIMS 1-3, 5, 9, 11, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §112, FIRST PARAGRAPH - POSSESSION

Claims 1-3, 5, 9, 11, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the claimed subject matter. The Examiner states that the claims are directed to a genus of polypeptides that comprise a protease domain or catalytically active portion thereof of a type-II membrane-type serine protease (MTSP) from any source including any or all recombinants, variants and mutants of MTSP or MTSP1. The Examiner alleges that the claims thus are allegedly drawn to polypeptides having any structure and allegedly are thus a structurally diverse genus. The Examiner states that the description of solely structural features present in all members of the genus is not sufficient to be representative of the attributes and features of the entire genus. The Examiner alleges that there is insufficient written description because the specification allegedly teaches only four species, and the Examiner contends that the disclosure of four species is not enough to describe the whole genus, and alleges that there is no evidence on record of the relationship between the structure of the serine protease domains of SEQ ID NOs. 2, 4, 6 and 11 and the structure of any or all MTSP polypeptides or a catalytically active portion of an MTSP polypeptide. The rejection is respectfully traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application. The relevant law and a discussion of the Patent Office Guidelines are set forth in the previous responses of record in this application, which are incorporated by reference herein. Briefly, the Federal Circuit has discussed the application of the written description requirement of the first paragraph of 112 to claims in the field of biotechnology. *See University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398, 1406 (Fed. Cir. 1997). The court explained that:

> In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus . . . a generic statement such as "vertebrate insulin or "mammalian insulin without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is.

The court also stated that "[a]written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or]chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." *Id.* at 1567, 43 U.S.P.Q.2d at 1405. Finally, the court addressed the manner by which a genus of might be described. "A description of a genus of may be achieved by means of a recitation of a representative number of defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." *Id.*

The Federal Circuit also has addressed the written description requirement in the context of biotechnology-related subject matter in *Enzo Biochem. Inc. v. Gen-Probe*, 296 F.3d 1316, 63 USPQ2d (BNA) 1609 (Fed. Cir. 2002). The *Enzo* court adopted the standard that:

> the written description requirement can be met by 'showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.'

The court in *Enzo* adopted its standard from the Written Description Examination Guidelines. The Guidelines apply to proteins as well as nucleic acid molecules.

It is well-settled that the written description requirement of 35 U. S. C. §112, first paragraph, can be satisfied without express or explicit disclosure of a later-claimed invention. *See, In re Herschler*, 591 F.2d 693, 700-01, 200 USPQ 711, 717 (CCPA 1979):

> "The claimed subject matter need not be described *in haec verba* to satisfy the description requirement. It is not necessary that the application describe the claim limitations exactly, but only so clearly that one having ordinary skill in the

> pertinent art would recognize from the disclosure that appellants invented processes including those limitations." (citations omitted).

See also Purdue Pharma L. P. v. Faulding, Inc., 230 F.3d 1320, 56 USPQ2d 1481 (Fed. Cir. 2000). In order to satisfy the written description requirement, the disclosure as originally filed does not have to provide *in haec verba* support for the claimed subject matter at issue.

The Claims

Claim 1 is directed to a substantially purified single-chain polypeptide consisting of a protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, where the MTSP protease domain or catalytically active fragment thereof has serine protease activity as a single chain. Claim 2, 3, 5, 9, 11, 19, 20, 34-36, 40-42, 113 and 114 ultimately depend from claim 1 and are directed to various embodiments thereof.

Analysis

In setting forth the rejection, the Examiner urges that the specification does not set forth what specific structural or physical features define the claimed polypeptides and argues that one skilled in the art could not predict the structure and function of the claimed polypeptides that comprise a protease domains or catalytically active portion thereof of any or all MTSP polypeptides. The Examiner alleges that the genus of claim 1 and its dependent claims are structurally diverse because it encompasses any polypeptide that comprises a catalytically active protease domains of any or all MTSP or all MTSP1 and has serine protease activity. The Examiner states that the claims are drawn to polypeptides having any structure and are thus drawn to a genus encompassing species having substantial variation (Office Action, page 8) and that description of solely structural features present in all members of the genus is not sufficient to be representative of the attributes and features of the entire genus (see Office Action, page 9). It respectfully is submitted that this is not correct.

Claim 1 and claims depending from claim 1 recite a substantially purified single-chain polypeptide consisting of a protease domain of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, where the MTSP protease domain or catalytically active fragment thereof has serine protease activity as a single chain. It is respectfully submitted that the instant application adequately describes the claimed polypeptides to demonstrate possession of the claimed subject matter at the time of the effective filing date of each claim. As is discussed in more detail below, to satisfy the written description requirement, one need not provide an example of every species encompassed by a

claim. It is sufficient to provide identifying characteristics, including structural and physical characteristics, functional characteristics coupled with known or disclosed correlation with structural characteristics to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; *see University of California v. Eli Lilly*, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Further, the standard is an objective one, based on what one of skill in the art would recognize in the disclosure. *In re Gosteli*, 872 F.2d at 1012.

First, the structural feature, a single chain protease domain, is present in all members of the genus and is the defining and requisite property thereof. The specification clearly describes this feature and demonstrates possession thereof.

Second, as discussed above, the instant application discloses that the isolated protease domains of members of the type II transmembrane protease (MTSP) family possess protease activity as a single-chain polypeptide. The application notes and describes known MTSPs and identifies the protease domains thereof. In addition, the application identifies and provides heretofore unknown MTSPs and provide full-length proteases and also the isolated protease domains. For example, the application exemplifies and describes **MTSP1** (or matriptase), **MTSP3, MTSP4** and **MTSP6, corin** (accession nos. AF133845 and AB013874; see, Yan *et al.* (1999) J. Biol. Chem. 274:14926-14938; Tomia *et al.* (1998) J. Biochem. 124:784-789; Uan *et al.* (2000) Proc. Natl. Acad. Sci. U.S.A. 97:8525-8529; SEQ ID Nos. 61 and 62 for the human protein); **enteropeptidase** (also designated enterokinase; accession no. U09860 for the human protein; see, Kitamoto *et al.* (1995) Biochem. 27: 4562-4568; Yahagi *et al.* (1996) Biochem. Biophys. Res. Commun. 219:806-812; Kitamoto *et al.* (1994) Proc. Natl. Acad. Sci. U.S.A. 91:7588-7592; Matsushima *et al.* (1994) J. Biol. Chem. 269:19976-19982; see SEQ ID Nos. 63 and 64 for the human protein); **human airway trypsin-like protease** (HAT; accession no. AB002134; see Yamaoka *et al.* J. Biol. Chem. 273:11894-11901; SEQ ID Nos. 65 and 66 for the human protein); **hepsin** (accession nos. M18930, AF030065, X70900; Leytus *et al.* (1988) Biochem. 27: 11895-11901; Vu *et al.* (1997) J. Biol. Chem. 272:31315-31320; and Farley *et al.* (1993) Biochem. Biophys. Acta 1173:350-352; SEQ ID Nos. 67 and 68 for the human protein); **TMPRS2** (see, Accession Nos. U75329 and AF113596; Paoloni-Giacobino *et al.* (1997) Genomics 44:309-320; and Jacquinet *et al.* (2000) FEBS Lett. 468: 93-100; SEQ ID Nos. 69 and 70 for the human protein) **TMPRSS4** (see, Accession No. NM 016425; Wallrapp *et al.* (2000) Cancer 60:2602-2606; SEQ ID Nos. 71 and 72 for the human protein); and **TADG-12** (also designated MTSP6, see SEQ ID Nos. 11 and 12; see International PCT application No. WO

00/52044, which claims priority to U.S. application Serial No. 09/261,416). The MTSPs are described in the specification as a known family of proteases. The instant application provides additional new members and also discloses for the first time that the protease domain of any member of this family is active as an isolated single-chain polypeptide that contains only the protease domain. Since the MTSP family and members thereof are known, and the application provides numerous examples (see *e.g.*, list above), and their sequences and locus of the protease domains are known or can be determined as taught in the application, it is clear that the inventors had possession of a genus of protease domains.

As described in the application, all of these proteins are members of the MTSP family and contain a protease domain, whose locus is known or that can be readily identified as described in the application. The application states:

> The protease domains as provided herein are single-chain polypeptides, with an N-terminus (such as IV, VV, IL and II) generated at the cleavage site (generally having the consensus sequence R↓VVGG, R↓IVGG, R↓IVNG, R↓ILGG, R↓VGLL, R↓ILGG or a variation thereof; an N-terminus R↓V or R↓I, where the arrow represents the cleavage point) when the zymogen is activated. To identify the protease domain an RI should be identified, and then the following amino acids compared to the above noted motif.
>
> The protease domains generated herein, however, do not result from activation, which produces a two chain activated product, but rather are single chain polypeptides with the N-terminus include the consensus sequence ↓VVGG, ↓IVGG, ↓VGLL, ↓ILGG or ↓IVNG or other such motif at the N-terminus. As shown herein, such polypeptides, although not the result of activation and not double-chain forms, exhibit proteolytic (catalytic) activity.

Hence the specification teaches a genus of peptides and teaches that the protease domain of each can be isolated and that it has proteolytic activity. Many of these polypeptides are known polypeptides, and the locus of the protease domain was known. What was not known, however, is that the protease domain can be isolated and that it exhibits proteolytic activity as a single chain. The application clearly and unequivocally demonstrates and exemplifies such single chain protease domains. As discussed in detail below, the specification provides relevant identifying characteristics, including structural and physical characteristics of an MTSP protease domain or catalytically active portion thereof, provides a number of exemplary protease domains, and the specification also directs those skilled in the art to exemplary art that describes common structural features shared by the protease domain of transmembrane serine proteases. There should be no doubt that the applicant had possession of the genus of isolated single chain protease domains of the MTSP family at the time of filing.

1. The specification provides (and the claims recite) relevant identifying characteristics of MTSP polypeptides, including structural and physical characteristics of serine proteases

An objective standard for determining whether a disclosure complies with the written description requirement is an affirmative answer to the query: "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed?" *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989). The instant specification defines a genus of polypeptides as claimed such that one of skill in the art would recognize that genus. Recognition thereof is sufficient to evidence Applicant's possession of the claimed subject matter.

The polypeptides of claim 1 and claims dependent thereon recite a specific structural domain of an MTSP, the protease domain or a catalytically active portion thereof. The specification describes the structural features of the protease domain, including additional structure, such as the catalytic triad, primary specificity pocket, oxyanion hole and conserved motifs (*e.g.*, see page 19, lines 3-21). The claims also recite as an additional structural limitation that the polypeptide is a single chain. The specification teaches, and it is known in the art, that serine proteases are expressed as an inactive single-chain zymogen that are subsequently activated by cleavage of the single chain to form a two-chain polypeptide that contains the protease domain disulfide bonded to amino acids upstream of the protease domain. What was not known in the art is that an isolated single-chain form of the protease domain exhibits proteolytic function. Hence the art does not provide isolated protease domains, but provides full-length MTSP proteases and identifies cleavage sites for activation cleavage. The instant application teaches that activation cleavage is not required, so that polypeptides that contain only the protease domain exhibit activity.

Further as noted above, the application provides the amino acid sequences of numerous members of the family:

> **MTSP1** (or matriptase), **MTSP3, MTSP4** and **MTSP6, corin** (accession nos. AF133845 and AB013874; see, Yan *et al.* (1999) J. Biol. Chem. 274:14926-14938; Tomia *et al.* (1998) J. Biochem. 124:784-789; Uan *et al.* (2000) Proc. Natl. Acad. Sci. U.S.A. 97:8525-8529; SEQ ID Nos. 61 and 62 for the human protein); **enteropeptidase** (also designated enterokinase; accession no. U09860 for the human protein; see, Kitamoto *et al.* (1995) Biochem. 27: 4562-4568; Yahagi *et al.* (1996) Biochem. Biophys. Res. Commun. 219:806-812; Kitamoto *et al.* (1994) Proc. Natl. Acad. Sci. U.S.A. 91:7588-7592; Matsushima *et al.* (1994) J. Biol. Chem. 269:19976-19982; see SEQ ID Nos. 63 and 64 for the human protein); **human airway trypsin-like protease** (HAT; accession no. AB002134; see Yamaoka *et al.* J. Biol.

> Chem. 273:11894-11901; SEQ ID Nos. 65 and 66 for the human protein); **hepsin** (accession nos. M18930, AF030065, X70900; Leytus *et al.* (1988) Biochem. 27: 11895-11901; Vu *et al.* (1997) J. Biol. Chem. 272:31315-31320; and Farley *et al.* (1993) Biochem. Biophys. Acta 1173:350-352; SEQ ID Nos. 67 and 68 for the human protein); **TMPRS2** (see, Accession Nos. U75329 and AF113596; Paoloni-Giacobino *et al.* (1997) Genomics 44:309-320; and Jacquinet *et al.* (2000) FEBS Lett. 468: 93-100; SEQ ID Nos. 69 and 70 for the human protein) **TMPRSS4** (see, Accession No. NM 016425; Wallrapp *et al.* (2000) Cancer 60:2602-2606; SEQ ID Nos. 71 and 72 for the human protein); and **TADG-12** (also designated MTSP6, see SEQ ID Nos. 11 and 12; see International PCT application No. WO 00/52044, which claims priority to U.S. application Serial No. 09/261,416).

Hence the specification provides complete structure features of at least 10 members of the family, as well as directions for identifying a protease domain and also for identifying and isolating new members of the family.

As discussed above, the application states:

> The protease domains as provided herein are single-chain polypeptides, with an N-terminus (such as IV, VV, IL and II) generated at the cleavage site (generally having the consensus sequence R↓VVGG, R↓IVGG, R↓IVNG, R↓ILGG, R↓VGLL, R↓ILGG or a variation thereof; an N-terminus R↓V or R↓I, where the arrow represents the cleavage point) when the zymogen is activated. To identify the protease domain an RI should be identified, and then the following amino acids compared to the above noted motif.
>
> The protease domains generated herein, however, do not result from activation, which produces a two chain activated product, but rather are single chain polypeptides with the N-terminus include the consensus sequence ↓VVGG, ↓IVGG, ↓VGLL, ↓ILGG or ↓IVNG or other such motif at the N-terminus. As shown herein, such polypeptides, although not the result of activation and not double-chain forms, exhibit proteolytic (catalytic) activity.

Hence the application teaches how to identify proteinase domains within the known sequences of amino acids.

2. Members of the MTSP family of serine proteases were well known at the time of filing

The standard for evaluating written description is objective, based on what one of skill in the art would recognize in the disclosure. *In re Gosteli*, 872 F.2d at 1012. Hence, evaluation of written description takes into account the knowledge of one of skill in the art with regard to the particular subject matter.

The claimed polypeptides are single chain polypeptides consisting of the protease domain of a type-II MTSP or catalytically active portion thereof. As discussed above, the full length sequences as well as the locus of the protease domains of a variety of MTSP serine protease family members were known at the time of filing the priority application, and the

instant application identifies several new members of the family and provides their protease domains. For example, Yan *et al.* ((1999) J. Biol. Chem. 274:14926-14938) and Tomia *et al.* ((1998) J. Biochem. 124:784-789) describe the serine protease corin. Kitamoto *et al.* ((1995) Biochem. 27: 4562-4568), Yahagi *et al.* ((1996) Biochem. Biophys. Res. Commun. 219:806-812), Kitamoto *et al.* ((1994) Proc. Natl. Acad. Sci. U.S.A. 91:7588-7592), and Matsushima *et al.* ((1994) J. Biol. Chem. 269:19976-19982) describe enteropeptidase. Yamaoka *et al.* ((1998) J. Biol. Chem. 273:11894-11901) describes human airway trypsin-like protease. Leytus *et al.* ((1988) Biochem. 27: 11895-11901), Vu *et al.* ((1997) J. Biol. Chem. 272:31315-31320) and Farley *et al.* ((1993) Biochem. Biophys. Acta 1173:350-352) describe hepsin. Paoloni-Giacobino *et al.* ((1997) Genomics 44:309-320) and Jacquinet *et al.* ((2000) FEBS Lett. 468: 93-100) TMPRS2. Wallrapp *et al.* ((2000) Cancer 60:2602-2606) describes TMPRSS4. International PCT application No. WO 00/52044 (which claims priority to U.S. application Serial No. 09/261,416) describes TADG-12. Each of these references is incorporated by reference in the instant application.

Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) and Yan *et al.* ((1999) *J. Biol. Chem. 274*:14926-35)) teach that serine proteases are a family of proteins that can be distinguished from many other types of proteins and enzymes because they have highly conserved structures. For example, a cleavage site at the N-terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding were identified as highly conserved features in serine proteases (see, *e.g.*, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*) In addition, a correlation between retention of the catalytic triad and retention of serine protease activity was demonstrated and known in the art at the time of filing. For example, Craik *et al.* ((1987) Science 237:909-13), Sprang *et al.* ((1987) Science 237:905-09), Carter *et al.* ((1988) *Nature 332:*564-68) and Bachovchin *et al.* ((1981) *Proc. Natl Acad. Sci. 78*: 7323-26)) teach that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad. Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) teaches that serine proteases are synthesized a single-chain zymogens that are proteolytically activated to become active two-chain forms (*e.g.*, see page 18235, col. 2, first full paragraph).

3. One of skill in the art would recognize Applicant's possession of the claimed subject matter

To satisfy the written description requirement, the issue is not whether the specification literally lists all of the possible MTSP protease domains and variants thereof

that fall within the scope of the claims, but whether one of skill in the art in view of the specification would recognize that applicant had provided a genus of single-chain polypeptides with the recited protease domain structure given the disclosure of the instant application. As noted above, the application provides at least a dozen examples, and provides relevant structural and functional features that uniquely identify and specify the claimed genus of polypeptides. The specification teaches that those of skill in the art recognize common elements among MTSPs and the protease domains of MTSPs, and the specification teaches a number of conserved characteristics for the MTSPs. For example, see page 49, lines 3-15, which discloses:

> The MTSPs are a family of transmembrane serine proteases that are found in mammals and also other species that share a number of common structural features including: a proteolytic extracellular C-terminal domain; a transmembrane domain, with a hydrophobic domain near the N-terminus; a short cytoplasmic domain; and a variable length stem region containing modular domains. The proteolytic domains share sequence homology including conserved his, asp, and ser residues necessary for catalytic activity that are present in conserved motifs. The MTSPs are synthesized as zymogens, and activated to double chain forms by cleavage. It is shown herein that the single chain proteolytic domain can function *in vitro* and, hence is useful in *in vitro* assays for identifying agents that modulate the activity of members of this family. Also provided are family members designated MTSP3, MTSP4 and an MTSP6 variant.

The specification provides additional structural and functional characteristics of the various MTSPs. For example, the specification teaches that the MTSP family of proteases include a serine residue that is involved in the hydrolysis of proteins or peptides. The serine residue can be part of the catalytic triad mechanism, which includes a serine, a histidine and an aspartic acid in the catalysis, or can be part of the hydroxyl/ε-amine or hydroxyl/α-amine catalytic dyad mechanism, which involves a serine and a lysine in the catalysis (for example, see page 17, lines 24-30). Further the specification teaches, for example at page 19, lines 3-24, that:

> Exemplary MTSP proteins, with the protease domains indicated, are illustrated in Figures 1-3. Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains

> share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190, SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

The specification also directs those skilled in the art to exemplary art that describes common structural features shared by the transmembrane serine proteases (for example, see page 18, lines 1-15). Thus, the specification discloses and the art recognizes that there are common conserved elements among the protease domains of MTSPs, such as the active site triad, a primary specificity pocket and an oxyanion hole. **Furthermore, the Type II transmembrane serine proteases are a recognized genus of polypeptides.**

An adequate written description for a claimed genus only has to provide "relevant, identifying characteristics" of a representative number of species (MPEP §2163). The specification provides a number of examples of MTSP protease domains, explicitly and implicitly. As noted, the specification provides at least a dozen examples of MTSPs and isolated protease domains, including MTSP1, MTSP3, MSTP4 (2 splice variants) and MTSP6. As quoted above, the disclosure on pages 9-10 recites:

> Other MTSP protease domains of interest herein, particularly for use in *in vitro* drug screening proteolytic assays, include, but are not limited to: corin (accession nos. AF133845 and AB013874; see, Yan *et al.* (1999) J: Biol. Chem. 274:14926-14938; Tomia *et al.* (1998) J. Biochem. 124:784-789; Uan *et al.* (2000) Proc. Natl. Acad. Sci. U.S.A. 97:8525-8529; SEQ ID Nos. 61 and 62 for the human protein); enteropeptidase (also designated enterokinase; accession no. U09860 for the human protein; see, Kitamoto *et al.* (1995) Biochem. 27: 4562-4568; Yahagi *et al.* (1996) Biochem. Biophys. Res. Commun. 219:806-812; Kitamoto *et al.* (1994) Proc. Natl. Acad. Sci. U.S.A. 91:7588-7592; Matsushima *et al.* (1994) J. Biol. Chem. 269:19976-19982; see SEQ ID Nos. 63 and 64 for the human protein); human airway trypsin-like protease (HAT; accession no. AB002134; see Yamaoka *et al.* J. Biol. Chem. 273:11894-11901; SEQ ID Nos. 65 and 66 for the human protein); hepsin (see, accession nos. M18930, AF030065, X70900; Leytus *et al.* (1988) Biochem. 27: 11895-11901; Vu *et al.* (1997) J. Biol. Chem. 272:31315-31320; and Farley *et al.* (1993) Biochem. Biophys. Acta 1173:350-352; SEQ ID Nos. 67 and 68 for the human protein); TMPRS2 (see, Accession Nos. U75329 and AF113596; Paoloni-Giacobino *et al.* (1997) Genomics 44:309-320; and Jacquinet *et al.* (2000) FEBS Lett. 468: 93-100; SEQ ID Nos. 69 and 70 for the human protein) TMPRSS4 (see, Accession No. NM 016425; Wallrapp *et al.* (2000) Cancer 60:2602-2606; SEQ ID Nos. 71 and 72 for the human protein); and TADG-12 (also designated MTSP6, see SEQ ID Nos. 11 and 12; see International PCT application No. WO 00/52044, which claims priority to U.S. application Serial No. 09/261,416).

As noted in the quoted paragraph, sequences of these protease are provided. Also provided are sequences of MTSP3, the MTSPs 4, and MTSP6 (see page 52, lines 12-31):

> Specific sequences for the following human MTSPs and domains thereof are provided as follows: SEQ ID No. 3 MTSP3 nucleic acid sequence; SEQ ID No. 4 MTSP3 amino acid sequence; SEQ ID No. 5 MTSP4 nucleic acid encoding the protease domain; SEQ ID No. 6 MTSP4 amino acid sequence of the protease domain; SEQ ID No. 7 MTSP4-L nucleic acid sequence; SEQ ID No. 8 MTSP4-L amino acid sequence; SEQ ID No. 9 MTSP4-S nucleic acid sequence; SEQ ID No. 10 MTSP4-S amino acid sequence; SEQ ID No. 11 MTSP6 nucleic acid sequence; SEQ ID No. 12 MTSP6 amino acid sequence. SEQ ID No. 1 sets forth the nucleic acid sequence of the long form of MTSP1; SEQ ID No. 2 the encoded amino acid sequence; SEQ ID No. 49 sets forth the sequence of a protease domain of an MTSP1, and SEQ ID No. 50 the sequence of the encoded single chain protease domain thereof. Figures 1-3 depict the structural organization of the MTSP3, MTSP4 and MTSP6, respectively.
>
> In particular, exemplary protease domains include at least a sufficient portion of sequences of amino acids set forth as amino acids 615-855 in SEQ ID No. 2 (encoded by nucleotides 1865-2587 in SEQ ID No. 1; see also SEQ ID Nos. 49 and 50) from MTSP1 (matriptase), amino acids 205-437 of SEQ ID NO. 4 from MTSP3, SEQ ID No. 6, which sets forth the protease domain of MTSP4, and amino acids 217-443 of SEQ ID No. 11 from MTSP6.

Hence, the specification explicitly discloses at least a dozen MTSP family member proteases (matriptase, corin, enteropeptidase, human airway trypsin-like protease, hepsin, TMPRS2, TMPRSS4 and TADG-12) and provides specific nucleic acid sequences and amino acid sequences for exemplary species.

The specification states that the claimed single-chain polypeptide consists of an MTSP protease domain or catalytically active portion thereof that can be from any MTSP family, for example from a mammal, including human MTSP. For example, see page 8, line 30 through page 9, line 8, which recites:

> The protease domains provided herein include, but are not limited to, the single chain region having an N-terminus at the cleavage site for activation of the zymogen, through the C-terminus, or C-terminal truncated portions thereof that exhibit proteolytic activity as a single-chain polypeptide in *in vitro* proteolysis assays, of any MTSP family member, preferably from a mammal, including and most preferably human, that, for example, is expressed in tumor cells at different levels from non-tumor cells, and that is not expressed on an endothelial cell.

The specification provides methods for identification, production, isolation, synthesis and/or purification of MTSP protease domains. The specification states, for example, that MTSP3, MTSP4 and MTSP6 are isolated from any animal, particularly a mammal, and includes but are not limited to, humans, rodents, fowl, ruminants and other animals (see page 20, lines 21-23; page 21, lines 11-13; and page 21, lines 29-31, respectively). Alternative methods for

obtaining the MTSP protein than by directly isolating the MTSP protein also are provided. These include synthesis using genomic DNA, chemically synthesizing the gene sequence from a known sequence and making cDNA to the mRNA that encodes the MTSP protein, for example, and inserting the isolated nucleic acids into an appropriate cloning vector (for example, see pages 67-79).

The instant specification clearly describes structurally and functionally known MTSPs. The catalytic function of MTSPs is known in this art. The catalytically active purified single-chain form polypeptide including the protease domain of type II MTSPs or catalytically active portions thereof described in the instant application elicit their effect through these known functions of the protease domains of MTSPs. The activity of the claimed substantially purified single-chain polypeptide consisting of the protease domain of a MTSP or a catalytically active portion thereof is described and demonstrated for the exemplary polypeptides.

The specification also defines structural features and structure-function relationships that identify the claimed genus of polypeptides consisting of a protease domain or catalytically active portion thereof and having serine protease activity. Such description includes information regarding the tertiary structure. For example, the specification teaches the locus of the disulfide bonds, identifies the Cys residues that link the protease domain to the rest of the polypeptide, and teaches that the polypeptide includes at least one of the active site triad, primary specificity pocket and oxyanion hole. The specification states that the serine protease family of proteins shares a high degree of homology. Hence, other related proteins, such as MTSPs from other species, can be readily identified. The specification also teaches that the protease domain of a MTSP shares homology and structural features with the chymotrypsin/trypsin family protease domains. The previous response and the application establish that the application describes the MTSP family and describes identification and isolation of protease domains.

In addition, the specification also provides exemplary assays in which catalytic activity of the polypeptides can be tested (for example, see Examples 3 and 4). If necessary, one of skill in the art could test the polypeptides for catalytic activity using the assays provided or known to those of skill in art or to review the sequences to determine which possess the requisite protease domain structure in order to identify those that possess catalytic activity without undue experimentation.

In addition, the standard for evaluating written description is based on the knowledge of skill in the art. *In re Gosteli*, 872 F.2d at 1012. As discussed in detail above with respect to MTSPs and the protease domains thereof, the knowledge of one of skill in the art is and was high at the time of filing and before. The claimed polypeptides consist of an MTSP serine protease domain or catalytically active portion thereof. The protease domain was well known in the art and easily identified by identifying the activation cleavage site in the polypeptide.

The recited protease domain is a feature that one of skill in the art can use to identify the claimed polypeptides. As explained in detail above, the MTSP family was a known protein family; sequences of the full-length proteases were known, and numerous members of the family had been identified and characterized. The instant application provides several new members. The presently pending claims are directed to isolated single-chain protease domains, which the instant application teaches and demonstrates have activity as single chain polypeptides. The instant application provides the sequences of more than a dozen members of the family. Hence, the recitation in the claims that the polypeptides consist of a protease domain from an MTSP and are single-chain polypeptides indicates "with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass." Therefore, the claims and application satisfy the standard set in *Eli Lilly* and the requirements of 35 U.S.C. §112, first paragraph.

As explained, the activation cleavage site that borders the protease domain of an MTSP as well as the structural features of a protease domain were well-known in the art. One skilled in the art would recognize that applicant had possession of protease domains from MTSPs having activity as a single chain. The Examiner has failed to indicate why one of skill in the art, in view of the description in the specification of methods for preparing and testing polypeptides for activity and in view of the extensive knowledge of those of skill in the art, would be unable to recognize, upon reading the disclosure, that Applicant invented the claimed subject matter. The specification teaches that numerous members of the MTSP family are and were known, provides additional members, teaches how to identify and isolate protease domains as single chains and how to assess activity. One of skill in the art could, if needed, readily test any of the those polypeptides for catalytic activity.

Therefore, the combination of the disclosure of the specific chemical structures of at least a dozen species within the scope of the claims as well as teachings in the specification (and knowledge of those of skill in the art) of assays for testing for activity and the evidence

that those of skill in the art are very familiar with the serine protease structure and function renders it clear that one of skill in the art would recognize that applicant had possession of the claimed polypeptides. The generic "invention" devolves to a recognition that a single chain isolated form of the protease domain of members of this known family has protease activity. One of skill in the art would have recognized from reading the disclosure that Applicant had possession of this genus as well as numerous species thereof. This teaching and knowledge coupled with the ability to test for functional mutants with the assays provided for in the specification and known in the art demonstrates that Applicant sufficiently described and was in possession of the polypeptides as claimed, at the effective filing date(s) of the claims.

In light of Applicant's disclosure, one of skill in the art would have recognized from reading the application that Applicant provided single-chain polypeptides with the recited protease domain structure that possess serine protease activity. Given the fact that numerous members of the MTSP family were known at the time of filing, the features of the protease domain of serine protease polypeptides identified in the application and known in the art, coupled with the ability to test isolated single chain polypeptides for serine protease activity using assays provided in the application and known in the art, one of skill in the art would recognize that Applicant was in possession of the claimed subject matter at the effective filing date(s) of the claims.

IV. REJECTION OF CLAIMS 1-3, 5, 9, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §112, FIRST PARAGRAPH – Scope of Enablement

Claims 1-3, 5, 9, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 112, first paragraph, because the specification allegedly fails to describe the claimed subject matter in such a way as to enable one skilled in the art to make and use the claimed subject matter commensurate in scope with these claims. The Examiner states that the specification is enabling for a polypeptide that includes amino acids 615-855 of SEQ ID NO:2, amino acids 205-437 of SEQ ID NO:4, amino acids of SEQ ID NO:6 and amino acids 217-443 of SEQ ID NO:112. The Examiner alleges that the specification does not reasonably provide enablement for a polypeptide that comprises any protease domain of any type II membrane type serine protease or catalytically portion thereof that include variants. The Examiner alleges that predictability of which changes in a protein's amino acid structure can be tolerated requires a knowledge of and guidance with regard to the sequence as to which are tolerant to modification and which are conserved, and detailed knowledge of how the protein's structure relates to function. It is alleged that it would require undue experimentation for one of skill in the art to

make such modified polypeptides with an expectation of success because the result of such modifications in unpredictable. It is further alleged that the claimed polypeptides encompass a large number of polypeptides and that the specification does not provide sufficient guidance on the nature of the changes that can be tolerated such that the proteins retain activity. In response to Applicant's arguments in the previous Response, evidencing the extensive knowledge in the art with respect to serine proteases, the instant Office Action argues that these arguments are not persuasive because the specification allegedly does not establish which specific amino acids in the protein's sequence can be modified such that the modified polypeptide continues to have proteolytic activity. The Examiner alleges that while the art may teach the general structure of MTSP and conserved amino acid sequences, protease domains, X-ray crystal structure and other attributes, such teachings "will not reduce the burden of undue experimentation on those of ordinary skill in the art." Therefore, the Office Action concludes, it would require undue experimentation to produce claimed polypeptides.

This rejection is respectfully traversed. As discussed below, above and previously, notwithstanding the disclosure of new proteases and individual protease claims, the instant application discloses and claims a generic invention: isolated single-chain protease domains from MTSPs. The MTSP protein family is a well-known and well characterized family of proteins and numerous members are known and disclosed in the application and in the art. In addition, the application provides new members. The specification teaches identification, preparation and isolation of protease domains and those of skill in the art, in view of the application, readily can identify and isolate a protease domain from any MTSP. Hence there is no reason to limit the claims to particular species of the family, when one of skill in the art, in light of the disclosure, can identify all members of the genus.

Relevant Law

The discussion of the relevant law from previous responses is incorporated herein.

Analysis

Application of the Factors Enumerated in *In re Wands*

It respectfully is submitted that analysis of enablement requires consideration of all of the "Wands Factors" and that focusing on one or two of the factors is a misapplication of the law. Applicant has discussed application of the "Wands Factors" in the previous responses, and such discussions are incorporated herein by reference. It would not require undue experimentation to isolate single-chain protease domains from any MTSP polypeptide. Further, it would not require undue experimentation to make modifications thereto. The

Examiner admits that enzyme isolation techniques and recombinant and mutagenesis techniques are known in the art, and that it is routine in the art to screen for substitutions or modifications, including multiple substitutions and multiple modifications as encompassed by the instant claims (see Office Action, page 11). As discussed in detail below, a consideration of the factors enumerated in *In re Wands* demonstrates that the application, in conjunction with what was known to one of skill in the art, teaches how to make and use the subject matter as claimed without undue experimentation.

Breadth of the Claims

Claim 1 is directed to an isolated substantially **purified single-chain polypeptide consisting of a protease domain** of a type-II membrane-type serine protease (MTSP) or a catalytically active fragment thereof as a single chain, wherein the protease domain or catalytically active fragment thereof has serine protease activity as a single chain. Claims 2, 3, 19, 20, 34-36, 40-42, 113 and 114 ultimately depend from claim 1 and recite additional features and specific family members.

Claims 2 and 3 are directed to polypeptides of claim 1 where the MTSP is not expressed on endothelian cells (claim 2) or is not expressed on normal endothelial cells *in vivo* (claim 3). Claims 19 and 20 are directed to polypeptides of claim 1 where a free Cys in the protease domain is replaced with another amino acid and the polypeptide retains serine protease activity. Claim 34 recites particular polypeptides within the scope of claim 1. Claims 35 and 36 are directed to a conjugates including a polypeptide of claim 1 and a targeting agent linked to the protein directly or via a linker. Claims 40-42 are directed to a solid support including two or more polypeptides of claim 1 linked thereto either directly or via a linker. Claims 113 and 114 are directed to a solid support including two or more polypeptides of claim 12 linked thereto either directly or via a linker.

Level of Skill

The level of skill in this art is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Teachings of the Specification

As discussed above and previously, the specification teaches that MTSP polypeptides constitute a recognized well-known and well characterized family of serine proteases. For example, page 18, lines 1-23 recites:

As used herein, "transmembrane serine protease (MTSP)" refers to a family of transmembrane serine proteases that share common structural features as described herein (see, also Hooper *et al.* (2001) J. Biol. Chem.276:857-860). Thus, reference, for example, to "MTSP" encompasses all proteins encoded by the MTSP gene family, including but are not limited to: MTSP1, MTSP3, MTSP4 and MTSP6, or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. Other MTSPs include, but are not limited to, corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4. Sequences of encoding nucleic molecules and the encoded amino acid sequences of exemplary MTSPs and/or domains thereof are set forth in SEQ ID Nos. 1-12, 49, 50 and 61-72. The term also encompass MTSPs with conservative amino acid substitutions that do not substantially alter activity of each member, and also encompasses splice variants thereof. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Of particular interest are MTSPs of mammalian, including human, origin. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* Molecular Biology of the Gene, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224).

The specification teaches that a protease domain from an MTSP polypeptide is active as a single-chain polypeptide. Additionally, smaller fragments of the protease domain also are active as single-chain polypeptides (page 18, line 24-page 19, line 2):

As used herein, a "protease domain of an MTSP" refers to the protease domain of MTSP that is located within the extracellular domain of a MTSP and exhibits serine proteolytic activity. It includes at least the smallest fragment thereof that acts catalytically as a single chain form. Hence it is at least the minimal portion of the extracellular domain that exhibits proteolytic activity as assessed by standard assays in vitro assays. Those of skill in this art recognize that such protease domain is the portion of the protease that is structurally equivalent to the trypsin or chymotrypsin fold.

The specification further teaches that MTSP protease domains can vary in sequence but that these proteins retain a conserved structure as well as sequence identity to identified MTSP proteins exemplified in the application. For example, see page 19, lines 3-24, which recites:

Exemplary MTSP proteins, with the protease domains indicated, are illustrated in Figures 1-3, Smaller portions thereof that retain protease activity are contemplated. The protease domains vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enteropeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, e.g., Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary

> for activity are present in conserved motifs. The activation site, which results in the N-terminus of second chain in the two chain forms is has a conserved motif and readily can be identified (see, e.g., amino acids 801-806, SEQ ID No. 62, amino acids 406-410, SEQ ID No. 64; amino acids 186-190, SEQ ID No. 66; amino acids 161-166, SEQ ID No. 68; amino acids 255-259, SEQ ID No. 70; amino acids 190-194, SEQ ID No. 72).

The application describes the protease domain of a number of MTSP family members including MTSP1, MTSP3, MTSP4 and MTSP6 as well as HAT, corin, enteropeptidase, TMPRSS4 and TMPRSS2. Identification of the protease domain from an MTSP region merely requires identification of the activation cleavage site and several other structural features as outlined in the specification and known in the art. The locus of the protease domain in the known MTSP family members is known, and the instant application provides protease domains from several other family members. A comparison of sequence identity among family members (see, *e.g.*, Figure 4 of the application) reveals that the protease domains share conserved sequences, including the catalytic triad of His, Asp and Ser residues and their surrounding conserved motifs. Additionally, the specification demonstrates that MTSP protease domains can have a reasonable amount of sequence variation and yet retain serine protease activity. MTSP1, MTSP3, MTSP4 and MTSP6 protease domains share about 40% sequence identity with each other. The specification teaches that each of these protease domains is an example of an MTSP protease domain that has activity in the single chain form.

The specification also teaches additional modifications. For example, see page 26, lines 13-25, which recites:

> Hence smaller portions of the protease domains, particularly the single chain domains, thereof that retain protease activity are contemplated. Such smaller versions will generally be C-terminal truncated versions of the protease domains. The protease domains vary in size and constitution, including insertions and deletions in surface loops. Such domains exhibit conserved structure, including at least one structural feature, such as the active site triad, primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a single chain portion of an MTSP, as defined herein, but is homologous in its structural features and retention of sequence of similarity or homology the protease domain of chymotrypsin or trypsin. Most significantly, the polypeptide will exhibit proteolytic activity as a single chain.

The specification teaches that included in the conserved features of MTSP protease domain polypeptides is a catalytic triad as well as the activation cleavage site, which defines the terminus of the protease domain polypeptides when they are isolated as single chain polypeptides.

The specification explains that beyond such conserved features the polypeptides are tolerant of modification. The specification explains that such modifications can be effected

using numerous methods known in the art. For example, at page77, line 17 through page 78, line 11, the specification states:

> A variety of modifications of the MTSP proteins and domains are contemplated herein. An MTSP-encoding nucleic acid molecule can be modified by any of numerous strategies known in the art (Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated in vitro. In the production of the gene encoding a domain, derivative or analog of MTSP, care should be taken to ensure that the modified gene retains the original translational reading frame, uninterrupted by translational stop signals, in the gene region where the desired activity is encoded.
>
> Additionally, the MTSP-encoding nucleic acid molecules can be mutated in vitro or in vivo, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further in vitro modification. Also, as described herein muteins with primary sequence alterations, such as replacements of Cys residues and elimination of glycosylation sites are contemplated. Such mutations may be effected by any technique for mutagenesis known in the art, including, but not limited to, chemical mutagenesis and in vitro site-directed mutagenesis (Hutchinson *et al.*, J. Biol. Chem. 253:6551-6558 (1978)), use of TAB® linkers (Pharmacia). In one embodiment, for example, an MTSP protein or domain thereof is modified to include a fluorescent label. In other specific embodiments, the MTSP protein is modified to have a heterofunctional reagent, such heterofunctional reagents can be used to crosslink the members of the complex.

The specification exemplifies variation in MTSP sequences. For example the specification provides exemplary MTSP1, MTSP3, MTSP4 and MTSP6 sequences. The specification explains that MTSP1 and MTSP3 amino acid sequences have about 43% identity with each other (for example, see page 162, lines 1-2). The specification also discloses that MTSP1 and MTSP4 have about 37% amino acid sequence identity (for example, see page 167, lines 25-29). The specification also teaches that MTSP4 and MTSP6 share about 60% amino acid sequence identity (for example, see page 172, lines 4-9). The specification teaches that each of the protease domains of these MTSP family members is active as single chain that contains only the protease domain or a smaller catalytically active portion of the protease domain (see, for example at page 20, lines 1-6). Hence, the specification teaches that MTSP protease domains share about 40%-60% and greater sequence identity and are active as single chain polypeptides.

The specification teaches additional modifications of the MTSP polypeptides. For example, the specification explains that for each individual MTSP, the polypeptides can include about 60% amino acid sequence identity with the exemplified MTSP. Such modified

polypeptides exhibit serine protease activity as single chain polypeptides. The specification provides exemplary modifications including conservative amino acid substitution (for example, see page 10, lines 3-13) and modifications of cysteine residues and/or of glycosylation sites (for example, see page 78, lines 1-7). The specification also discloses that non-natural amino acids can be introduced as a substitution or addition in the MTSP polypeptides (for example, see page 79, lines 10-21).

Knowledge of those of skill in the art

As discussed above, at the time of filing of the application and before, those of skill in the art were very familiar with serine proteases generally, and with the MTSP family in particular. The family was known as was the locus of the protease domain. What was absent was any understanding or recognition that an isolated single chain protease domain would have activity; hence, such was never isolated. In view of the instant application teaching that such protease domains have activity as single chains, the skilled artisan can readily isolate any protease domain as a single chain of an MTSP. Nothing more need be known regarding the requisites for activity.

Notwithstanding this, there was a large body of literature directed to serine proteases and there was general understanding of their structures and requisites for activity (see for example, Hooper *et al. J. Biol. Chem. 276*:857-860, Nienaber *et al.* (2000) *J. Biol. Chem.* 275:7239-48; Sommerhoff *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96:10984-91; Lu *et al.* (1999) J. Mol. Biol. 292:361-73; Xu *et al.* (2000) *J. Biol. Chem.* 275:378-385, Lin *et al.*(1999) *J. Biol. Chem. 274*: 18231-36 and Bryan (2000) *Biochem. Biophys. Acta 1543*:200-03). These references detail the existing crystal structures, structural comparisons and structural similarities of serine proteases.

This extensive knowledge also is evidenced, for example, in the application as filed and in the literature made of record in the submitted Information Disclosure Statements. As noted in the application, the Type II Serine Proteases family (TTSPs), also referred to as MTSPs, were known (for example, see pages 4-5). Serine proteases are a family that can be distinguished from many other types of proteins and enzymes because they have highly conserved structures (see *e.g.*, Lin *et al.* (1999) *J. Biol. Chem. 274*:18231-36 and Yan *et al.* (1999) *J. Biol. Chem. 274*:14926-35). Moreover, it was known at the time of filing, there is a known correlation between retention of the catalytic triad and retention of serine protease activity. Hence, available to one of skill in the art was the knowledge that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad (see for example, Carter *et al.* (1988) *Nature 332:*564-68, Sprang *et al.* (1987)

Science 237:905-09, Craik *et al.* (1987) Science 237:909-13 and Bachovchin *et al.* (1981) *Proc. Natl Acad. Sci. 78*: 7323-26). In addition, other features were identified at the time of filing as highly conserved features in serine proteases including a cleavage site at the N-terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding (see for example, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*) Hence, the requisites for retention of serine protease activity are well-known and characterized and were available at the effective filing date of the claimed subject matter. Hence, a wide variety of structural information on serine proteases was well-known in the art.

Furthermore, the instant claims only require identification of the protease domain of an MTSP, and its isolation as a single chain polypeptide. A number of MTSPs were known and the locus of the protease domain identified. Those of skill in the art can readily identify the protease domain region in an MTSP, and, if necessary test it for the protease activity.

The methods and guidance for comparing amino acid sequences to generate and confirm sequences with sequence identity to an MTSP polypeptide sequence such as SEQ ID NOS: 2, 4, 6 and 12 was available in the art at the time of filing the instant application. As described in the instant specification, computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:2444 were available. Other available programs include the GCG program package (Devereux, J., *et al.* , Nucleic Acids Research 12(I):387 (1984)), BLASTP, BLASTN, and FASTA (Atschul *et al.*, J Molec Biol 215:403 (1990); *Guide to Huge Computers*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo *et al.* (1988) SIAM J Applied Math 48:1073). In addition, methods for generating nucleotide and protein sequence variation were widely available in the art. Thus, one of skill in the art could use such programs with a serine protease sequence, for example, to align the sequence and identify the structural features of importance for retention of activity and use the methods for generating sequence variation to make the identified protein variants. The Examiner states that enzyme isolation techniques and recombinant and mutagenesis techniques are well known.

Methods for assaying protease activity including protease specificity, level of activity and response to inhibitors was well known in the art (see, for example, Lu *et al.* (1999) J. Mol. Biol. 292:361-73; Xu *et al.* (2000) *J. Biol. Chem.* 275:378-385). Methods for high throughput assays and detection were also widely available (See generally, *High Throughput Screening: The Discovery of Bioactive Substances* (Devlin, Ed.) Marcel Dekker, 1997;

Sittampalam *et al., Curr. Opin. Chem. Biol.*, 1:384-91 (1997); and Silverman *et al., Curr. Opin. Chem. Biol.*, 2:397-403 (1998)). Hence, the amount of knowledge of those of skill in the art was extensive and the requisite structural and functional features required for protease activity was well known.

The Examiner states that the specific amino acid positions within a protein's sequence where amino acid modification can be made with a reasonable expectation of success in obtaining the desired activity are limited in any protein and the result of such modifications is unpredictable. Applicant respectfully disagrees in the case of the family of serine proteases. The previous response, the art and the application establish that serine proteases are well known in the art and the structural requirements for activity are known and that the instantly claimed polypeptides share sequence homology with the chymotrypsin/trypsin family for which tertiary structures are known. For example, it was known in the art that serine protease activity could be retained in a serine protease by retaining the conserved structure of the catalytic triad (see *e.g.*, Craik *et al.*, Science 237:909-13 (1987), Sprang *et al.*, Science 237:905-09 (1987), Carter *et al.*, *Nature 332:*564-68 (1988) and Bachovchin *et al. Proc. Natl Acad. Sci. 78*: 7323-26 (1981)). Other highly conserved features in serine proteases also were known to the skilled artisan. These include a cleavage site at the N-terminus of the protease domain, a substrate specificity pocket in the protease domain and conserved cysteines that participate in disulfide bonding (see for example, Figure 4 and page 18235 of Lin *et al.* and Figure 2 and page 18236 of Yan *et al.*). The specification also provides exemplary assays for testing catalytic activity of the polypeptides using routine experimental analysis techniques and also provides descriptions of how to assess percentage identity and teaches that these techniques were well known in the art. The specification also teaches conserved characteristics among serine proteases. Furthermore, the MTSPs are a known family of serine proteases, and the protease domain of any member can be readily identified.

The Examiner states that recombinant and mutagenesis techniques and enzyme isolation techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). Thus, routine techniques can be used to identify or synthesize modified MTSP serine protease domains. If needed, one of skill in the art can test polypeptides for catalytic activity by routine experimentation using the assays provided in the specification or known to those of skill in art. Furthermore, the instant claims are directed to single chain protease domains of MTPSs, which are known. Issues regarding modification and requisites therefor are irrelevant.

Contrary to the Examiner's position, one of skill in the art would conclude that such a description in the specification constitutes a sufficiently detailed description of identifying characteristics of the claimed subject matter consistent with *Enzo* (supra), particularly in view of the fact that these proteins are members of the well-characterized serine protease family of proteins. In fact, related family members are in the art, which is of record and are described in the application.

Working Examples

The application provides working examples that demonstrate each of the features of the claimed polypeptides. For instance, the Examples provide detailed guidance for identifying and isolating MTSP protease domains. Example 1 describes the cloning of and identification of MTSP3 based on its sequence similarity with MTSP1. Example 2 describes the identification and cloning of two MTSP4 polypeptides, MTSP4-S and MTSP4-L. Example 3 describes the identification and cloning of an MTSP6 polypeptide based on sequence similarity to MTSP4. In each case, an MTSP polypeptide sequence is identified that includes a protease domain with a cleavage site and a catalytic triad (see, e.g., Figure 4). As noted, for example, in Example 1, identification of MTSP3 as a serine protease required only 43% sequence identity. Similarly, Example 2 demonstrates that 37% sequence identity with MTSP1 was sufficient to identify MTSP4.

The Examples demonstrate additional features of the claimed polypeptides. For example, Examples 1, 2, 3 and 6 each demonstrate the expression of MTSP polypeptides in normal and tumor tissues. The working examples further demonstrate that each of the MTSP polypeptides, having, for example, 37-43% sequence identity, is active as a single chain protease domain.

The Examples demonstrate expression of single chain protease domains. For example, Example 1 describes the cloning of MTSP3 into an expression vector and expressing it in *E. coli*. The example describes the purification of the protein and the serine protease activity of the single chain protease domain using a variety of substrates. Examples 4 and 5 describe additional expression vector cloning techniques for *Pichia pastoris* expression for MTSP 3, 4 and 6. Example 5 provides a detailed example of a serine protease assay for the expressed MTSP6 single chain protease domain. Examples 6 and 7 provide a detailed description of the cloning, expression and purification of an MTSP1 single chain protease domain. Example 8 provides detailed serine protease assays for MTSP1. Additionally, Example 1 demonstrates that additional sequence variation can be introduced into single chain protease domains of an

MTSP, such as a cysteine to serine change, without altering serine protease activity. Hence, the examples demonstrate the ability of one of skill in the art to isolate and express MTSP single chain polypeptides that include the protease domain without additional regions of MTSP sequence. The examples further demonstrates that one of skill in the art can identify MTSP sequences with 37-43% sequence identity that share common features of an MTSP and are active as single chain polypeptides.

As discussed above, the application provides the sequences and identities of at least a dozen MTSP family members and describes identification of the protease domain. One of skill in the art can readily isolate a protease domain as a single chain from any MTSP family member.

Predictability

The predictability at issue herein is whether one of skill in the art could isolate protease domains from MTSP family members and variants thereof, including serine protease family members that are single chain protease domains that have at least about 95% sequence identity with an MTSP1 protease domain that includes the sequence of amino acids set forth as residues 615-855 in SEQ ID NO:2, as amino acids 205-437 in SEQ ID NO:4, as the amino acid residues in SEQ ID NO:6 or as amino acids 217-443 of SEQ ID NO:12, or variants that differ in only 5% of the residues. Applicant respectfully submits that one of skill in the art, given the instant disclosure, could predictably make such polypeptides, because the MTSP family is well known and the sequences of exemplary new family members, as well as known members, are provided in the application. One of skill in the art could readily make minor amino acid variation there, and, if needed, test such polypeptide variants for serine protease activity.

In contrast to the allegations of "unpredictability" set forth in the Office Action, the specification and the knowledge in the art evidence many factors of *predictability* with respect to MTSP polypeptide variants. First, the specification provides more than a dozen exemplary polypeptides. These are defined chemical structures from which one of skill in the art is given a reference point. As explained above, included among exemplary polypeptides are MTSP1, MTSP3, MTSP4-S, MTSP4-L and MTSP6, which share about 40% sequence identity. The specification demonstrates, however, that these MTSP polypeptides share conserved features including a protease domain with a catalytic triad and N-terminal activation cleavage site. Furthermore, the specification teaches isolation of the protease domains as single chains and demonstrates that they possess proteolytic activity.

Second, the specification delineates structural and functional features of the protein. These features identify key regions and residues that one of skill in the art would know to conserve in order to retain serine protease activity. These features also provide reference points for alignments with other known serine proteases. These features also allow one of skill in the art to make further structure-function correlations, again providing predictable correlations of regions and residues to conserve or change. As evidenced by the references cited in the specification and in the Information Disclosure Statements of record in this application, a large body of knowledge pertaining to structure-function relationships of serine proteases was known in the art. In addition, the specification provides exemplary assays to assess serine protease activity, including a variety of substrates for MTSP activity. Additional serine protease assays were available in the art at the time of filing the instant application. One of skill in the art could readily and routinely test any MTSP family member protease domain or a variant thereof for serine protease activity as a single chain protease.

As taught in the specification as well as evidenced by the art of record, maintenance of the catalytic triad is sufficient to retain serine protease activity. Therefore, one of skill in the art could make and generate MTSP family member protease domains as well as variants of MTSP protease domains with at least 95% identity. Serine protease activity of these variants could easily and routinely be confirmed using the assays provided in the application and known in the art. The routine manipulations to identify and isolate an MTSP protease domain as a single chain as well as to generate minor variants thereof, *e.g.* selecting non-catalytic triad residues and aligning variant sequences to confirm at least about 95% identity, are not unpredictable.

The experimentation necessary to make and use MTSP polypeptides, as described above, is routine. "Enablement is not precluded by the necessity for some experimentation such as routine screening. Experimentation needed to practice the invention must not be undue experimentation. 'The key word is *undue*, not experimentation.' " *In re Wands*, 858 F.2d at 737-38 (quoting *In re Angstadt*, 537 F.2d at 504; emphasis added; additional internal citations omitted). The Examiner admits that enzyme isolation techniques and recombinant and mutagenesis techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). The art related to serine proteases also demonstrates that such experimentation is not undue. For example, Pearson *et al.* ((1997) *Cabios Invited Review 13(4)*: 325-32) explains that serine proteases share a conserved catalytic site, the catalytic

triad. In addition, trypsin-like serine proteases have several diagnostic motifs throughout the protein including a conserved protein fold and anti-parallel β barrel structures that contribute to the function of the protease. Pearson *et al.* states that one could recognize proteins that have protease activity based on these conserved structures. Hence, generation of variants with serine protease activity is routine because one of skill in the art can use such conserved features as a guide for designing the location of variations to maintain these features. In addition, Cheah *et al.* ((1990) *J. Biol. Chem. 265*:7180-7187) provides a demonstration of the predictability of generating variants of serine proteases based on an exemplary sequence. The authors use known structural and functional information about trypsin-like serine proteases to obtain mutations in a rhinovirus 3C protease with predicted functional phenotypes. Thus, the art available at the time of filing, and before, demonstrates that one of skill in the art could make variants of a serine protease in a predictable manner.

Therefore, one of skill in the art could make protease domains as single chains from an MTSP family member and also generate variants of MTSP polypeptides, including those with at least about 95% identity. Activity of the single chain protease domains and variants thereof could easily and routinely be confirmed using the assays provided in the application and known in the art. The routine manipulations to generate an MTSP single chain protease domains are not unpredictable.

The instant application identifies MTSP polypeptides that possess serine protease activity as a single chain. Such demonstration of single chain activity had not been demonstrated before the instant application. The application provides adequate description to demonstrate that a common feature among the MTSP family members is the activity of a single chain form that includes the protease domain or catalytically active portions thereof in the absence of other MTSP portions. The application provides exemplary MTSP's that share about 40% sequence identity and possess such features. Therefore, the specification demonstrates that by following the teachings of the application, one of skill in the art can predictably identify, make and use substantially purified polypeptides consisting of an MTSP protease domain or catalytically active fragment thereof having serine protease activity as a single chain.

The amount of experimentation required

There is nothing of record to suggest that production or use of any of the claimed polypeptides would require development of new procedures or excessive experimentation. Protein extraction, purification and synthesis methods have been used for decades. As discussed above, assays for evaluating activity of the polypeptides are taught in the

specification and are known in the art. Such assays are routine in this art and do not require excessive experimentation. The Examiner states that recombinant and mutagenesis techniques and enzyme isolation techniques are known and that it is routine to screen for multiple substitutions or multiple modifications as encompassed by the instant claims (see Office Action, page 11). Hence, the claimed polypeptides can be synthesized, isolated and characterized using routine testing, and, if necessary, one of skill in the art can test polypeptides for catalytic activity by routine experimentation using the assays provided in the specification or known to those of skill in art. Applicant notes that "a considerable amount of experimentation is permissible, if it is merely routine . . ." *In re Wands* 858 F.3d 731, 737

Conclusion

In light of the breadth of the claims, the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, and the fact that it is predictable to identify protease domains in MTSP family members and prepare single chain forms thereof as well as variants thereof, including those with at least 95% sequence identity, it would not require undue experimentation for one of skill in the art to make and use polypeptides with the features as claimed. Accordingly, a consideration of the factors enumerated above leads to the conclusion that, based on the disclosure in the specification, undue experimentation would not be required to make and use polypeptides as instantly claimed.

V. REJECTION OF CLAIMS 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 AND 114 UNDER 35 U.S.C. §102(a)

Claims 1-3, 5, 11-13, 19, 20, 34-36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 102(a) as anticipated by Takeuchi *et al.* (Proc. Natl. Acad. Sci. USA 96: 11054-11061 (1999)) because Takeuchi *et al.* allegedly discloses a polypeptide comprising a fragment consisting of a serine protease domain that is 100% identical to amino acids 615-855 of SEQ ID NO:2, purifying a polypeptide comprising a fragment consisting of a serine protease domain that is identical to amino acids 615-855 of SEQ ID NO:2, that its polypeptide is not expressed on normal endothelial cells, is of human origin, consists essentially of the protease domain having catalytic activity and is expressed on tumor cells. The Examiner also alleges that the reference discloses a solid support that includes a polypeptide comprising a fragment consisting essentially of a serine protease domain that is identical to amino acids 615-855 of SEQ ID NO:2. This rejection is respectfully traversed.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir, 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). It is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference. *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

"Rejections under 35 U.S.C. §102 are proper only when the claimed subject matter is identically disclosed or described in the 'prior art' . . . the [r]eference must clearly and unequivocally disclose the claimed compound or direct those skilled in the art to the compound without *any* need for picking, choosing, and combining various disclosures not directly related to each other by the teachings in the cited references. Such picking and choosing may be entirely proper when making a rejection of a §103, obviousness rejection, where the applicant must be afforded an opportunity to rebut with objective evidence any inference of obviousness which may arise from the *similarity* of the subject matter which he claims to the prior art, but it has no place in the making of a §102, anticipation rejection." (Emphasis in original). *In re Arkey, Eardly, and Long*, 455 F.2d 586, 172 USPQ 524 (CCPA, 1972).

The Claims

See related section above.

Disclosure of Takeuchi *et al.*

Takeuchi *et al.* discloses a polypeptide that contains 855 amino acids and is designated MT-SP1. This protein has sequence identity with the full-length MTSP1 set forth as SEQ ID NO:2 of the instant application. Takeuchi *et al.* discloses an expression vector that includes nucleic acid encoding the protease domain ***plus*** the pro-domain (see page 11055, left col., third full paragraph). Takeuchi *et al.* discloses expressing its polypeptide in *E. coli* X-90 (page 11055, col. 2, 4th full paragraph). Takeuchi *et al.* discloses that its expression vector

includes the mature protease domain, which is ***two-chain molecule***, and a small portion of the pro-domain and was designed to over-express the sequence encoding a polypeptide containing amino acids 596-855 with a His-tag fusion to produce as a construct Met-Arg-Gly-Ser-His_6-aa596-855 (page 11055, column 2, third full paragraph). Takeuchi *et al.* identifies the locus of the MT-SP1 protease domain (amino acids 615-855; see Fig. 4, page 11058) in the longer protein, but does not disclose its isolation as a single chain that consists only of the protease domain. Takeuchi *et al.* discloses that the pro-domain region is disulfide bonded to the protease domain of its construct (page 11055, column 2, third full paragraph). Takeuchi *et al.* discloses that amino acids Cys 604 and Cys 731 are disulfide bonded (see for example, at page 11060, col.1) and that the pro-domain is disulfide bonded to the protease domain (see Figure 4). Takeuchi *et al.* does not disclose, teach or suggest isolation of a single chain form of the protease domain.

Analysis

Takeuchi *et al.* does not anticipate the claimed subject matter for the following reasons.

1. The claimed polypeptide consists of a protease domain or catalytically active portion thereof

An element of the pending claims is that the isolated substantially purified polypeptide consists of a protease domain or a smaller catalytically active portion of the protease as a single chain. The polypeptides disclosed by Takeuchi *et al.* include additional peptide portions from its MT-SP1. For example, Figure 1 of Takeuchi *et al.* depicts the predicted protein sequence of its full length MT-SP1. The full-length MT-SP1 polypeptide disclosed by Takeuchi *et al.* includes LDLR repeats (453-487) and CUB domains (213-339) in addition to the protease domain (see FIG. 4). Hence, it is not a polypeptide consisting of a protease domain or a smaller catalytically active portion of the protease domain.

Takeuchi *et al.* also discloses a polypeptide construct that includes the protease domain, a His-tag and *a portion of the pro-domain*, and refers to this construct as its "purified protease domain." Takeuchi *et al.* discloses that its "purified protease domain" includes the His-tag sequence, stating that a monoclonal antibody directed against the N-terminal Arg-Gly-Ser-His_4 epitope is immunoreactive with its purified protein (see page 11058). Takeuchi *et al.* also discloses that part of its construct has an amino acid sequence containing amino acids 615-855 (numbering as set forth in FIG. 1), and that its polypeptide construct is a His-tag fusion product that contains amino acids 596-855 (as set forth in FIG. 1). Hence, the polypeptide construct disclosed by Takeuchi *et al.* includes a sequence of 19 amino acids (a

portion of the pro-domain) from the MTSP other than the protease domain. Thus, it does not disclose an isolated single-chain polypeptide consisting of the protease domain.

Takeuchi *et al.* discloses that the pro-domain region is disulfide bonded to the protease domain (see page 11058, col. 1 and page 11060, col. 1, first paragraph) and remains bonded to the protease domain after activation (page 11058, lines 8-9). Hence, the purified His-tagged protease domain of Takeuchi *et al.* includes MTSP portions other than the protease domain. Furthermore, this polypeptide is a two-chain polypeptide. Thus, Takeuchi *et al* does not disclose a single chain polypeptide consisting of a protease domain or a catalytically active portion of the protease domain.

2. The claimed polypeptide is an isolated single chain polypeptide

Another element of the claimed subject matter is that the polypeptide that consists of protease domain or a smaller catalytically active portion thereof and is a single-chain polypeptide. Takeuchi *et al.* discloses that its polypeptide includes the pro-domain and that the pro-domain is cleaved during auto-activation, resulting in a protease domain disulfide bonded to a pro-domain resulting in a two-chain form. Takeuchi *et al.* discloses that the pro-domain remains disulfide bonded to the protease domain after purification (see page 11058). Thus, Takeuchi *et al.* does not disclose an ***isolated substantially purified single-chain*** polypeptide consisting of a protease domain or catalytically active portion thereof.

Takeuchi *et al.* discloses that the His-tag portion of its protein (Met-Arg-Gly-Ser-His_6-aa596-855) is cleaved during activation (page 11057, col. 2, last paragraph), which would produce the polypeptide aa596-855. As discussed above, amino acids 596-614 are part of the pro-domain and are not part of the protease domain. Takeuchi *et al.* discloses that under non-reducing conditions the pro-domain is disulfide bonded to the protease domain. Takeuchi *et al.* discloses analysis of its protein using SDS/PAGE to assess activation cleavage. Under the conditions of SDS/PAGE, the disulfide bonding of the protease domain to the pro-domain would be eliminated, producing two separate chains. Because of its size, the liberated pro-domain chain migrates through the gel, and appears to be outside of the visual range of the gels shown in Fig. 6. Takeuchi *et al.* does not disclose isolating the polypeptide of the lower band in Fig. 6A from the gel. Hence, Takeuchi *et al.* does not disclose an isolated, substantially purified single-chain polypeptide consisting of a protease domain or a smaller catalytically active portion thereof that has serine protease activity as a single-chain.

3. Conclusion

Applicant respectfully submits that merely pointing out the locus that corresponds to the protease domain in the full-length protein does not constitute disclosure of ***an isolated single chain protease domain.*** Takeuchi *et al.* does not disclose, teach or even suggest isolating a single chain polypeptide consisting of only amino acids 615-855. Takeuchi *et al.* specifically teaches inclusion of the pro-domain to produce a two-chain polypeptide. The fact that one can point to a longer polypeptide and note the locus of a domain thereof, does not constitute isolation of the domain. Takeuchi *et al.* simply does not disclose an **isolated single chain polypeptide consisting of amino acids 615-855 of its MT-SP1 polypeptide.** The isolated polypeptides disclosed by Takeuchi *et al.* contain additional MT-SP1 polypeptide. Thus, Takeuchi *et al.* does not disclose a purified single chain polypeptide consisting of a protease domain or a smaller catalytically active portion thereof that has serine protease activity as a single chain. Thus, Takeuchi *et al.* does not disclose every element of claim 1. Therefore, Takeuchi *et al.* does not anticipate claim 1 nor any claim dependent thereon.

REBUTTAL TO EXAMINER'S ARGUMENTS

1. Mischaracterization of Applicant's argument in previous response

Applicant respectfully submits that the Examiner has mischaracterized applicant's arguments in the previous response. The Examiner alleges that

> Even applicants state that [Takeuchi] et al. discloses "that its protease domain has an amino acid sequence containing amino acids 615-855" (Remarks page 36) and that "Takeuchi et al. discloses that its polypeptide includes the pro-domain and that the pro-domain is cleaved during auto-activation, resulting in a protease domain" (page 37).

The Examiner has taken the comments out of context and has not reproduced the whole argument as presented. The full text of the cited paragraphs states:

> Takeuchi *et al.* also discloses a polypeptide construct that includes the protease domain, a His-tag and *a portion of the pro-domain*, and refers to this construct as its "purified protease domain." Takeuchi *et al.* discloses that its "purified protease domain" includes the His-tag sequence, stating that a monoclonal antibody directed against the N-terminal Arg-Gly-Ser-His_4 epitope is immunoreactive with its purified protein (see page 11058). Takeuchi *et al.* also discloses that its protease domain has an amino acid sequence containing amino acids 615-855 (numbering as set forth in FIG. 1), and that its polypeptide construct is a His-tag fusion product that contains amino acids 596-855 (as set forth in FIG. 1). Hence, the polypeptide construct disclosed by Takeuchi *et al.* includes a sequence of 19 amino acids (a portion of the pro-domain) from the MTSP other than the protease domain. Takeuchi *et al.* discloses that this pro-domain region is disulfide bonded to the protease domain (see page 11058, col. 1

> and page 11060, col. 1, first paragraph) and remains bonded to the protease domain after activation (page 11058, lines 8-9). Hence, the purified His-tagged protease domain of Takeuchi *et al.* includes MTSP portions other than the protease domain.
>
> ...
>
> Another element of the claimed subject matter is that the polypeptide contains the protease domain or a smaller catalytically active portion thereof and is a single-chain polypeptide. Takeuchi *et al.* discloses that its polypeptide includes the pro-domain and that the pro-domain is cleaved during auto-activation, resulting in a protease domain disulfide bonded to a pro-domain resulting in a two-chain form. Takeuchi *et al.* discloses that the pro-domain remains disulfide bonded to the protease domain after purification (see page 11058). Thus, Takeuchi *et al.* does not disclose an ***isolated substantially purified single-chain*** polypeptide that includes ***as the only portion from the MTSP*** a protease domain or catalytically active portion thereof.

Thus, Takeuchi *et al.* discloses that its "purified protease domain" includes amino acids 596-855 (as set forth in FIG. 1). Hence, the polypeptide construct disclosed by Takeuchi *et al.* includes a sequence of 19 amino acids (a portion of the pro-domain) in addition to the protease domain. Further, when the pro-domain is cleaved during auto-activation, the resulting polypeptide is protease domain disulfide bonded to a pro-domain resulting in a two-chain form. Takeuchi *et al.* discloses that the pro-domain remains disulfide bonded to the protease domain after purification. Hence, Takeuchi *et al.* does not disclose a polypeptide consisting of an MTSP protease domain having activity as a single chain. The purified "activated" polypeptide of Takeuchi *et al.* includes the pro-domain disulfide bonded to the protease domain. Therefore, the Examiner has mischaracterized applicant's arguments by including only a portion of the argument to recite that the "pro-domain is cleaved during auto-activation, resulting in a protease domain." The argument as set forth clearly states that pro-domain is cleaved during auto-activation, resulting in a protease domain that is disulfide bonded to a pro-domain resulting in a two-chain active form.

2. Single chain polypeptide

The Examiner alleges that a single chain polypeptide is one sequence of amino acids beginning with a carboxyl end and terminating with an amino end, where the amino acids are connected via peptide bonds. The Examiner alleges that, using this definition, even the full length MT-SP1 of Takeuchi *et al.* having disulfide bonds can be construed as a single chain polypeptide. Applicant respectfully submits that this does not address the element as claimed. The instant claims are directed to ***isolated single-chain polypeptides consisting of*** an MTSP protease domain or catalytically active fragment thereof, where the protease domain has activity as a single chain. As known in the art of serine proteases as evidenced by the art of record,

until the disclosure of the instant application, one of skill in the art understood MTSP serine proteases to be active only as *two chain* polypeptides. For example, Lu *et al.* (1997) *J. Biol. Chem.* 272: 31293-31300, discloses that as expressed, the MTSP polypeptide is an **inactive** single-chain zymogen. Cleavage of the single-chain MTSP results in the production of a two-chain polypeptide where the protease domain is covalently bonded to the upstream polypeptide sequence by a disulfide bond, which results in an active two-chain serine protease. Applicant respectfully submits that Takeuchi *et al.* teaches that its polypeptide is inactive as a single chain form, and is cleaved into a two chain form that has activity.

As discussed above and previously, the understanding in the art at the time of the filing of the priority application, was that proteases required an activation cleavage event that results in a two-chain molecule with the protease domain disulfide bonded to additional amino acids for activity. Takeuchi *et al.* states this. The claims are directed to isolated single-chain polypeptides consisting of the protease domain of an MTSP. No one had ever isolated such species of protease.

VII. THE REJECTION OF CLAIMS 1-3, 5, 10-14 AND 34 UNDER 35 U.S.C. §102(e)/103(a)

Claims 1-3, 5, 10-14 and 34 are rejected under 35 U.S.C. §102(e) as anticipated by O'Brien *et al.* or in the alternative obvious over O'Brien *et al.*, because it is alleged that the reference discloses a polypeptide with 100% identity to full-length MTSP1 as set forth in SEQ ID NO:2 of the instant application. It is further alleged that the polypeptide disclosed by O'Brien *et al.* inherently possess the features set forth in claims 2-3 and 6-9 of the instant application. The Office Action also alleges that the reference discloses a protease domain identified therein as SEQ ID NO:14 that is 100% identical to amino acids 615-855 of SEQ ID NO:2. The Examiner states that O'Brien *et al.* does not disclose purifying the protease yet the Office Action concludes that the disclosed molecules in O'Brien *et al.* anticipate the claimed subject matter

In the alternative, it is alleged that the claims are obvious over the claimed subject matter because O'Brien *et al.* teaches a method of expressing polypeptides in host cells. It also is alleged that the reference teaches that the protease domain could be released from the polypeptide and used as a diagnostic that has the potential for therapeutic intervention. Thus, the Office Action concludes that it would have been obvious to one of skill in the art to express the protease domain disclosed as SEQ ID NO:14 by O'Brien *et al.* and purify the polypeptide. It is alleged that the motivation to make such polypeptides is the disclosed use

as a diagnostic for therapeutic intervention. Further, it is alleged that one of ordinary skill in the art would have had a reasonable expectation of success since the expression of heterologous polypeptides was routine in the art and O'Brien *et al.* teaches how to express heterologous polypeptides. This rejection respectfully is traversed.

Relevant Law

With respect to anticipation, the relevant law is set out above. Addressing obviousness, in order to set forth a prima facie case of obviousness under 35 U.S.C. § 103:

(1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention *(ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and

(2) the combination of the cited references must actually teach or suggest the claimed invention.

Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.*, 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher." *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Under 35 U.S.C. §103, in order to set forth a case of prima facie obviousness, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. See, *e.g.*, *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1462, 221 U.S.P.Q.2d 481, 488 (Fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963).

The Claims

Claims 1-3, 5,10-13 and 34 are discussed above.

A. THE ANTICIPATION REJECTION

The disclosure of O'Brien *et al.*

O'Brien *et al.* discloses a protein identified therein as TADG-15, which is an MTSP1 variant, with a sequence of amino acids as set forth as SEQ ID NO:2. The reference also discloses a comparison of the amino acid sequence of the protease domain of TADG-15 (SEQ ID NO:14) with other serine protease catalytic domains (see Figure 2). O'Brien *et al.* discloses that TADG-15 is a highly over-expressed gene in tumors and suggests that TADG-15 is novel in its component structure of domains because it has a protease catalytic domain that could be released and used as a diagnostic and which has the potential for a target for therapeutic intervention (col. 15, lines 31-38). O'Brien *et al.* states that TADG-15 can be expressed in host cells, for example from a construct including SEQ ID NO:1 (the nucleic acid sequence encoding the full-length TADG-15) and/or chemically synthesized. O'Brien *et al.* discloses in Example 7 that quantitative PCR was performed using sense and antisense primers for TADG-15. O'Brien discloses in Example 9 the use of 20mers derived from the catalytic domain of TADG-15 as specific primers and using the specific sequence of the full domain of the catalytic site of TADG-15 as a probe for Northern blot analysis and using the sequence for the TADG-15 catalytic domain of the protease as a probe to screen Hela and ovarian tumor cDNA libraries. O'Brien *et al.* does not disclose, teach or suggest isolation of the protease domain as a single-chain polypeptide that ***consists only of the protease domain as a single chain.***

Analysis

O'Brien *et al.* does not anticipate any of the instant claims. As explained above, claim 1 and claims dependent thereon are not directed to a full-length MTSP polypeptide. The claims are directed to isolated single-chain polypeptide consisting of an MTSP protease domain or smaller catalytically active portion thereof. In addition, the claimed polypeptides are single-chain polypeptides. The polypeptides disclosed by O'Brien *et al.* do not possess all of these characteristics.

SEQ ID NO:2 disclosed by O'Brien *et al.* sets for the sequence of a full-length MTSP. It includes not only a protease domain, but additional MTSP residues, as evidenced by the disclosure shown in Fig. 2 of O'Brien *et al.* SEQ ID NO:2 of O'Brien *et al.* includes a cytoplasmic domain, a transmembrane domain, a CUB repeat, a ligand binding repeat of LDL

receptor-like domain in addition to the serine protease domain (see Figure 10). Hence, SEQ ID NO:2 is not a disclosure of an isolated substantially purified polypeptide consisting of a protease domain or a smaller catalytically active portion of the protease domain. Therefore, the disclosure of SEQ ID NO:2 by O'Brien *et al.* does not anticipate any of the instant claims.

Second, O'Brien *et al.* does not disclose the expression, isolation or purification of SEQ ID NO:14., which is a subset of the sequence of amino acids set forth as SEQ ID NO:2 in O'Brien *et al.* O'Brien *et al.* states that SEQ ID NO:14 shows the serine protease catalytic domain of TAGD-15. The only mention of SEQ ID NO: 14 in the disclosure of O'Brien *et al.* is in Figure 2 (it is not even described in the sequence listing). Figure 2 shows a sequence comparison of SEQ ID NO:14 with protease domains from other proteases, including hepsin, trypsin, chymotrypsin and tissue plasminogen activator. SEQ ID NO. 14 is a representation; it is not an isolated polypeptide.

Applicant respectfully submits that a comparison of sequences of protease domains is not a disclosure of an isolated, substantially purified single chain polypeptide consisting of an MTSP protease domain or smaller catalytically active portion thereof. The Examiner admits that O'Brien *et al.* does not purify the protein identified as SEQ ID NO: 14 (see Office Action, page 20). Further, O'Brien *et al.* does not disclose isolating a protein that has the amino acid sequence as set forth as SEQ ID NO:14. Setting forth the sequence of a domain in the polypeptide is not a disclosure of an isolated polypeptide. Thus, the disclosure of SEQ ID NO:14 by O'Brien *et al.* does not disclose every element of claim 1 and therefore does not anticipate the claimed subject matter.

The Examiner's statement that "a single chain polypeptide is one sequence of amino acids beginning with a carboxyl end and terminating with an amino end, where the amino acids are connected via peptide bonds" does not address the element as claimed. The instant claims are directed to ***isolated single-chain polypeptides, not a representation thereof.*** As known in the art of serine proteases as evidenced by the art of record, until the disclosure of the instant application, one of skill in the art understood MTSP serine proteases to be active only as *two chain* polypeptides. For example, Lu *et al.* (1997) *J. Biol. Chem.* 272: 31293-31300, discloses that as expressed, the MTSP polypeptide is an **inactive** single-chain zymogen. Cleavage of the single-chain MTSP results in the production of a two-chain polypeptide where the protease domain is covalently bonded to the upstream polypeptide sequence by a disulfide bond, which results in an active two-chain serine protease. Hence, one of ordinary skill in the art, in light of the knowledge of the art, would view O'Brien *et al.*

as disclosing a polypeptide sequence of a serine protease zymogen and would expect the active protease domain to be part of a two chain polypeptide. One of ordinary skill in the art would not view a sequence listing setting forth a particular domain without further disclosure as an isolated polypeptide.

To be an anticipatory reference, a reference must put one of ordinary skill in the art in possession of what is claimed. The disclosure of O'Brien *et al.* provides no disclosure of an isolated single chain protease domain. As noted, the instant application represents the first recognition that single-chain protease domains possess protease activity. Prior to the instant application, the protease domain as an isolated entity had not been prepared.

B. THE OBVIOUSNESS REJECTION

Differences Between the Claims and the Teachings of O'Brien *et al.*

As explained above, O'Brien *et al.* teaches a protein identified therein as TADG-15 with a sequence of amino acids as set forth as SEQ ID NO:2. The reference teaches only the expression of the full-length TADG-15 in host cells. The reference does not provide any teaching or suggestion of any forms of TADG-15 that possess serine protease activity. The reference provides the linear amino acid sequence of SEQ ID NO:14, the stated protease domain of TADG-15, but the reference provides no teaching or suggestion of how one of ordinary skill in the art could generate a single-chain polypeptide containing such sequence and no other MTSP sequence. O'Brien *et al.* does not teach or suggest isolation or purification of a protein having an amino acid sequence set forth as SEQ ID NO:14. As discussed, the understanding in the art was that activation cleavage to produce a polypeptide containing the protease domain disulfide bonded to a second chain was necessary for activity. Hence, there would have been no teaching or suggestion of an isolated protease domain.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness because of the following:

The teachings of O'Brien *et al.* singly or in combination with what is known in the art do not result in the instantly claimed compositions.

As discussed above, instant claim 1 is not directed to the full-length protease. Claim 1 and its dependent claims are directed to polypeptides consisting of a protease domain or a smaller catalytically active portion thereof, where the polypeptide is a single chain and where the protease domain or a smaller catalytically active portion thereof has serine protease activity as a single chain. O'Brien *et al.* fails to teach or suggest polypeptides that include all

of these features. O'Brien *et al.* provides no teachings or suggestions that an isolated single chain polypeptide consisting only of the protease domain as a single chain has any activity.

O'Brien *et al.* teaches a full-length TADG-15 polypeptide. TADG-15 shares sequence identity with MTSP1; hence TADG-15 (SEQ ID NO:2 of O'Brien *et al.*) is not a polypeptide consisting of a protease domain or a smaller portion of the protease domain. Further, there is no teaching or suggestion of smaller fragments of TADG-15 that are single-chain polypeptides and that retain serine protease activity. The smaller fragments of TADG-15 taught by O'Brien *et al.* are small antigenic fragments, of from 10-50 residues, that have only the property of binding to a TADG-15-specific antibody (see, for example at col. 9, lines 22-39). There is no teaching or suggestion that any such fragments of TADG-15 retains catalytic activity.

Additionally, although O'Brien *et al.* teaches a linear sequence of amino acids set forth as SEQ ID NO:14 that includes a sequence identified as the protease domain of TADG-15, it does not teach or suggest expressing or isolating a protein with an amino acid sequence as set forth as SEQ ID NO:14. The application does not teach or suggest any expression of SEQ ID NO:14. Nor does it teach or suggest how to make a polypeptide consisting of such a sequence. O'Brien *et al.* suggests that the protease catalytic domain of TADG-15 "could be released" and used as a diagnostic but does not teach or suggest exactly what form such released form would take nor that such a "released" protease domain would be a single-chain form nor would exhibit serine protease activity.

Although the Office Action alleges that one of ordinary skill in the art could routinely express heterologous proteins and therefore would have had a reasonable expectation of success to express the protease domain, there is no teaching in the cited art that suggests isolation of a single chain polypeptide that contains only the protease domain. Absent teaching or suggestion to make such single chain polypeptide, the expectation of success is irrelevant. The ordinarily skilled artisan first has to think of making such polypeptide before the issue of expectation of success is considered. In this instance, it is the instant application that teaches an isolated single chain polypeptide containing only the protease domain of an MTSP.

As discussed above, at the time of filing the instant application, one of skill in the art recognized that any active protease domain of an MTSP polypeptide was a two-chain polypeptide (for example, see Lu *et al.* (1997) *J. Biol. Chem.* 272: 31293-31300), with the protease domain disulfide bonded to another portion of the MTSP polypeptide. The literature at the time of filing the instant application taught that MTSP serine proteases are synthesized as inactive single-chain zymogens that are activated by cleavage, which forms two-chain

polypeptides. This two-chain structure was taught in the art to be critical for serine protease function. Hence, in the absence of the instant application, the art at the time of filing evidenced that single-chain serine protease polypeptides were inactive (zymogens); none were taught to consist only of the protease domain.

In light of the what was known in the art at the time of filing the original application, it would not have been obvious that a single-chain polypeptide consisting of an MTSP serine protease domain or catalytically active portion thereof would have protease activity. Without further teachings specifically for the generation of a single-chain polypeptide that consists of an MTSP protease domain or catalytically active portion thereof, one would not have been motivated to modify the polypeptide taught by O'Brien to separate the protease domain and purify the protease domain such that the resulting polypeptide only consists of the protease domain, nor would there have been any expectation that doing so would result in a polypeptide that possesses serine protease activity as a single chain. Thus, O'Brien *et al.*, alone or in combination with what is known in the art, does not teach or suggest the polypeptides of claim 1. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

REBUTTAL TO EXAMINER'S ARGUMENTS

1. Art teaches that serine proteases are active only as two chain polypeptides

The Examiner alleges that the applicant in the previous Response stated that "as expressed, the MTSP polypeptide is an inactive single-chain zymogen" (Remarks page 42) and concludes that, according to the applicant, the full length MT-SP1 of O'Brien *et al.* is a single chain polypeptide and therefore, anticipates the claims. Applicant respectfully submits that the Examiner's interpretation of applicant's argument is incorrect. First it is noted that the claims do not read on a full-length polypeptide. Second, the full-length polypeptide is an inactive zymogen requiring activation cleavage. Hence it is unexpected that the an isolated single chain protease domain possesses activity.

Further, the paragraph from the previous response to which the Examiner refers states:

> The Examiner's statement that "a single chain polypeptide is one sequence of amino acids beginning with a carboxyl end and terminating with an amino end, where the amino acids are connected via peptide bonds" does not address the element as claimed. The instant claims are directed to an isolated single-chain polypeptide, not a representation thereof. As discussed in the previous Response, and known in the art of serine proteases as evidenced by the art of record, until the disclosure of the instant application, one of skill in the art understood MTSP serine proteases to be active only as *two chain* polypeptides. For example, Lu *et al.* (1999) *J. Biol. Chem.* 272: 31293-300, discloses that as

> expressed, the MTSP polypeptide is an inactive single-chain zymogen. Cleavage of the single-chain MTSP results in the production of a two-chain polypeptide where the protease domain is covalently bonded to the upstream polypeptide sequence by a disulfide bond, which results in an active serine protease. Hence, one of ordinary skill in the art, in light of the knowledge of the art, would view O'Brien *et al.* as disclosing a polypeptide sequence of a serine protease zymogen and would expect the protease domain to be part of a two chain polypeptide. One of ordinary skill in the art would not view a sequence ID setting forth a domain without further disclosure as an isolated polypeptide.

Lu *et al.* (the correction citation for which is Lu *et al.*, *J. Biol. Chem.* 272: 31293-31300 (1997)) teaches that enteropeptidase is synthesized as a single-chain precursor of 1035 amino acid residues that require proteolytic activation and that cleaving results in the active enteropeptidase, which is a disulfide-linked two-chain polypeptide (see page 31293, col. 2). Lin *et al.* ((1999) *J. Biol. Chem. 274*:18231-36) also teaches that serine proteases are synthesized as single-chain zymogens that are proteolytically activated to become active two-chain forms (*e.g.*, see page 18235, col. 2, first full paragraph). O'Brien *et al.* also teaches that as expressed, its MTSP polypeptide is an inactive single-chain zymogen. None of O'Brien *et al.*, Lu *et al.* or Lin *et al.* teaches or suggests that the protease domain is active as a single chain. Each of the references specifically teaches that only by cleaving the zymogen single chain form of the polypeptide to form a two-chain activated molecule is catalytic activity observed. Applicant specifically argued that :

> As discussed in the previous Response, and known in the art of serine proteases as evidenced by the art of record, until the disclosure of the instant application, one of skill in the art understood MTSP serine proteases to be active only as *two chain* polypeptides. For example, Lu *et al.* (1997) *J. Biol. Chem.* 272: 31293-300, discloses that as expressed, the MTSP polypeptide is an inactive single-chain zymogen. Cleavage of the single-chain MTSP results in the production of a two-chain polypeptide where the protease domain is covalently bonded to the upstream polypeptide sequence by a disulfide bond, which results in an active serine protease. Hence, one of ordinary skill in the art, in light of the knowledge of the art, would view O'Brien *et al.* as disclosing a polypeptide sequence of a serine protease zymogen and would expect the protease domain to be part of a two chain polypeptide.

Therefore, the Examiner's allegation that, according to the applicant, the full length MT-SP1 of O'Brien *et al.* is a single chain polypeptide and therefore, anticipates the claimed invention is contradictory to the argument set forth in the previous responses. The instant claims include as an element that the single-chain polypeptide has serine protease activity. Applicant's arguments clearly state that that the single-chain zymogen forms of serine proteases were known in the art to **not** have activity, and that the zymogens

required cleavage of the single-chain zymogen **to form a two-chain form** in order for the polypeptide to exhibit serine protease activity. Hence, a single-chain zymogen form of a serine protease does not anticipate any of the pending claims.

Inherency is not applicable to the question of obviousness.

The Examiner alleges that it would have been obvious to one having ordinary skill in the art to express the protease domain of SEQ ID NO:14 and to purify the polypeptide because the reference suggest its use as a diagnostic which has the potential for a target for therapeutic intervention. The Examiner was not persuaded by the argument that the skilled artisan, lacking the teachings of the instant specification, would not have had a reasonable expectation of success to express the protease domain as an active single-chain because the art evidences that a single-chain polypeptide would not have been expected to have protease activity. The Examiner states that that the polypeptide disclosed by O'Brien *et al.* being a single-chained polypeptide is an [inherent] property of the polypeptide. This argument is misplaced. Any discussion of inherency has no place in a consideration of obviousness. The concept of inherency is not applicable to the question of obviousness. *In re Sporman*, 363 F.2d 444, 150 USPQ 449 (CCPA 1965). To refer to an unexpected property or parameter as inherent begs the question of whether the unexpected property rebuts *prima facie* obviousness. Obviousness and inherency are entirely different questions; that which may be inherent is not necessarily known and, therefore, is an indication of unobviousness (*In re Sporman*, 363 F.2d 444, 449, 150 USPQ 449, 452 (CCPA 1965; see, also *In re Naylor*, 360 F.2d 765, 152 USPQ 106 (CCPA 1966); *In re Adams*, 356 F.2d 998, 148 USPQ 742 (CCPA 1966); and *In re Shetty*, 566 F.2d 81, 195 USPQ 753 (CCPA 1977)).

It is the instant application that provides the teaching to prepare an isolated single-chain protease domain; there is no teaching or suggestion for doing so in the prior art. Hence, the fact that if one were to prepare such single chain polypeptide, it would have activity is irrelevant, and, as in *In re Sporman*, begs the question of unobviousness. It is the teaching for making the single chain protease domain that is unobvious.

As discussed above, until the disclosure of the instant application, it was known in the art of serine proteases that MTSP serine proteases are active only as *two chain* polypeptides. As taught in the art (for example, see Lu *et al.* (1997) *J. Biol. Chem.* 272: 31293-300), the MTSP polypeptide is expressed as an inactive single-chain zymogen. Cleavage of the single-chain zymogen form of MTSP results in the production of a two-chain polypeptide in which the protease domain is covalently bonded to the upstream polypeptide sequence by a disulfide

bond, which results in an active serine protease. Hence, it would not have been obvious to one of ordinary skill in the art, in light of the knowledge of the art, that a substantially purified polypeptide consisting of an MTSP serine protease domain or catalytically active fragment thereof would have serine protease activity as a single-chain.

VIII. THE REJECTION OF CLAIMS 35, 36 40-42, 113 AND 114 UNDER 35 U.S.C. §103(a)

Claims 35, 36, 40-42, 113 and 114 are rejected under 35 U.S.C. § 103(a) as being unpatentable over O'Brien *et al.* (U.S. Patent No. 5,972,616) because O'Brien *et al.* allegedly teaches a polypeptide identified as SEQ ID NO:2 therein with identity to MTSP1 of the instant application. It is alleged that the reference teaches making fragments of SEQ ID NO:2, linking the fragments to a polypeptide and linking such polypeptides to solid supports. This rejection is respectfully traversed.

Relevant Law

See above.

The Claims

Claims 35 and 36 are directed to conjugates that include polypeptides of claim 1 and a targeting agent. The instant conjugates have serine protease activity. Claims 40-42 are directed to solid supports that include two or more polypeptides of claim 1. Claims 113 and 114 are directed to solid supports that include two or more polypeptides of claim 12.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The teachings of O'Brien *et al.* do not result in the instantly claimed compositions.

Claims 35, 36, 40-42, 113 and 114 ultimately depend from claim 1. As explained in detail above, O'Brien *et al.* does not teach or suggest a polypeptide consisting of an MTSP protease domain or a smaller catalytically active portion thereof, where the polypeptide is a single chain and where the MTSP protease domain or a smaller catalytically active portion thereof has serine protease activity as a single chain. There is no teaching or suggestion in O'Brien *et al.* of fragmenting its MTSP into a polypeptide consisting of the protease domain, where the protease domain has activity as a single-chain polypeptide. O'Brien *et al.* does not teach or suggest that "releasing" the protease domain from it TAGD-15 polypeptide in order to use it as a "diagnostic" results in an active protease domain, much less a single-chain protease domain with serine protease activity. One of ordinary skill in the art at the time of filing the

instant application would not have expected a polypeptide consisting of the protease domain of an MTSP to have protease activity, because the teachings in the art was that a single-chain polypeptide was inactive, and that the active form of the enzyme was a two-chain form, which includes the protease domain disulfide bonded to amino acids upstream of the protease domain. Thus, O'Brien *et al.* does not teach or suggest every element of claim 1, nor does the reference provide motivation to do what applicant has done. Hence, claim 1 is nonobvious over O'Brien *et al.* and therefore claims 35, 36, 40-42, 113 and 114, which ultimately depend from claim 1, also are nonobvious over O'Brien *et al.*

VIII. REJECTION OF CLAIMS 19 AND 20 UNDER 35 U.S.C. §103(a)

Claims 19 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over O'Brien *et al.* (U.S. Patent No. 5,972,616) and Estell *et al.* in view of Takeuchi *et al.* because it is alleged that O'Brien *et al.* teaches a serine protease domain of an MTSP polypeptide but does not teach replacing free Cys residues with Ser residues, but Estell *et al.* in light of Takeuchi *et al.* allegedly cures this defect. The Examiner alleges that it was well known in the art that proteins form disulfide bonds through SH groups of Cys residues. It is alleged that Takeuchi *et al.* teaches that position 731 normally forms a disulfide bond with a Cys residue in the pro-protease domain. The Office Action alleges that Estell *et al.* teaches that Cys residues replaced with Ser residues decrease a polypeptide's susceptibility to oxidation. The Office Action concludes that it would have been obvious to one of ordinary skill in the art to replace a free Cys residue in the protease domain taught by O'Brien *et al.* with a Ser residue in order to enhance stability of the protein. It is alleged that there would have been a reasonable expectation of success because Estell *et al.* teaches that such changes successfully decrease a protein's susceptibility to oxidation.

This rejection is respectfully traversed.

Relevant Law

See related section above.

The Claims

See related section above.

Teachings of the Cited References

O'Brien *et al.* and Takeuchi *et al.*

The teachings of O'Brien *et al.* and Takeuchi *et al.* are discussed above.

Estell *et al.*

Estell *et al.* teaches a method for producing prokaryotic carbonyl hydrolase enzymes, including subtilisin, in recombinant host cells. The method includes introducing mutations into the enzyme sequence including those that exhibit oxidative stability. Amino acids that can be mutated for oxidative stability according to Estell *et al.* include replacing tryptophan, methionine, cysteine and lysine with an amino acid such as alanine or serine.

Analysis

It is respectfully submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of teachings of O'Brien *et al.* with the teachings of Estell *et al.*, and Takeuchi *et al.* does not result in the instantly claimed polypeptide

As a preliminary matter, the Examiner alleges that the applicant in the previous response argued that that the combination of the teachings of O'Brien *et al.* with the teachings of Estell *et al.*, and Takeuchi *et al.* does not result in the instantly claimed methods. Applicant respectfully submits that no arguments directed to methods were provided in the previous response. In the previous response, Applicant argued, in part, that:

> None of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.*, alone or in any combination, teaches or suggests polypeptides with the features set forth in claim 1: a single chain polypeptide that includes an MTSP protease domain or smaller portion thereof as the only MTSP portion of the polypeptide where the protease domain or smaller portion thereof has catalytic activity as a single chain. In view of the failure of the references, alone or in any combination, to teach or suggest the polypeptides of claim 1, the combination of the references does not teach or suggest the polypeptides of dependent claims 16, 18-20 and 137, which include all of the limitations of claim 1. Thus, the combination of the teachings of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.* does not render any of the claimed subject matter obvious. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

As noted in the traverse above, if an independent claim is non-obvious, then claims dependent thereon are also nonobvious. Combining the teachings of O'Brien *et al.* with the teachings of Estell *et al.* and Takeuchi *et al.* does not teach or suggest the polypeptides of claim 1, and therefore the combination also does not teach or suggest the polypeptides of claims 19 or 20, which ultimately depend from claim 1.

The Examiner states that O'Brien *et al.* does not teach or suggest a serine protease domain of an MTSP where a free Cys residue is replaced with a Ser residue. In addition, O'Brien *et al.* does not teach or suggest a polypeptide consisting of an MTSP protease domain or catalytically active fragment thereof. O'Brien *et al.* does not teach or suggest an MTSP

protease domain or catalytically active fragment thereof that has a free Cys residue. Further, as explained in detail above, O'Brien *et al.* does not teach or suggest a single chain polypeptide consisting of an MTSP protease domain or smaller portion thereof, where the single chain polypeptide has serine protease activity. Also, as discussed above, there is no teaching or suggestion in O'Brien *et al.* of fragmenting its MTSP into a polypeptide consisting of the protease domain, where the protease domain has activity as a single-chain polypeptide. O'Brien *et al.* does not teach or suggest that "releasing" the protease domain from its TAGD-15 polypeptide in order to use it as a "diagnostic" results in an active protease domain, much less a single-chain protease domain with serine protease activity. One of ordinary skill in the art at the time of filing the instant application would not have expected a polypeptide consisting of the protease domain of an MTSP to have protease activity, because the teachings in the art was that a single-chain polypeptide was inactive, and that the active form of the enzyme was a two-chain form, which includes the protease domain disulfide bonded to amino acids upstream of the protease domain. Thus, O'Brien *et al.* does not teach or suggest every element of claim 1, nor does the reference provide motivation to do what applicant has done.

Takeuchi *et al.* does not remedy these defects. As discussed above, Takeuchi *et al.* does not teach a single chain polypeptide consisting of an MTSP protease domain or smaller portion thereof where the protease domain or smaller portion thereof has catalytic activity as a single chain. Takeuchi *et al.* teaches a full-length MT-SP1 and a *two*-chain activated polypeptide that includes the protease domain disulfide bonded to the pro-domain. There is no teaching or suggestion by Takeuchi *et al.* to generate a single-chain polypeptide or that the protease domain as a single-chain without its disulfide bonded pro-domain portion would be catalytically active. Further, Takeuchi *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue. As the Examiner points out, Takeuchi *et al.* teaches that the cysteine residue at position 731 of SEQ ID NO:2 forms a disulfide bond with a cysteine residue in the pro-domain (see page 11060 top left paragraph and Figure 4). Takeuchi *et al.* teaches that this disulfide linkage with the pro domain localizes the active catalytic domain to the cell surface via a disulfide linkage (see page 11060 top left paragraph). As shown in Figure 4, Takeuchi *et al.* teaches that *every* cysteine residue of the protein is disulfide bonded. Thus, because all of the cysteine residues of the polypeptide of Takeuchi *et al.* are disulfide bonded, Takeuchi *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue.

Estell *et al.* also fails to remedy the defects of O'Brien *et al.* and Takeuchi *et al.* Estell *et al.* does not teach or suggest any polypeptides that have any MTSP portions. Hence, Estell *et al.* does not teach or suggest any of the polypeptides of claim 1, which are single chained polypeptides consisting of an MTSP protease domain or catalytically active portion thereof. Estell *et al.* does not teach or suggest an MTSP protease domain having a free Cys residue. Thus, although Estell *et al.* teaches that cysteine residues can be replaced in prokaryotic carbonyl hydrolase enzymes, there is no teaching or suggestion of how to arrive at the instantly claimed polypeptides with the particular features of claim 1 or dependent claims 19 and 20.

None of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.*, alone or in any combination, teaches or suggests polypeptides with the features set forth in claim 1: a single chain polypeptide consisting of an MTSP protease domain or smaller portion thereof where the protease domain or smaller portion thereof has catalytic activity as a single chain. In view of the failure of the references, alone or in any combination, to teach or suggest the polypeptides of claim 1, the combination of the references does not teach or suggest the polypeptides of dependent claims 19 or 20, which include all of the limitations of claim 1. Thus, the combination of the teachings of O'Brien *et al.*, Takeuchi *et al.* and Estell *et al.* does not render any of the claimed subject matter obvious. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

REBUTTAL TO EXAMINER'S ARGUMENTS

1. Disulfide binding in the protease domain of Takeuchi *et al.*

The Examiner alleges that, as shown in Fig. 4 of Takeuchi *et al.*, the Cys at position 830 is disulfide bonded to Cys at position 191 of the full length MTSP. Applicant submits that Takeuchi *et al.* teaches at page 11060, col. 1, first paragraph that:

> Based on homology to chymotrypsin, three disulfide bonds are predicted to form within the protease domain at Cys-44–Cys-58, Cys-168–Cys-182, and Cys-191–Cys-220 (Cys-643–Cys-657, Cys-776–Cys-790, and Cys-801–Cys-830), and a forth disulfide bond should form between the catalytic and the pro-domain Cys-122–Cys-1 (Cys-731 – Cys-604), as observed for chymotrypsin. This predicted disulfide with the pro domain suggests that the active catalytic domain should still be localized to the cell surface via a disulfide linkage.

Thus, Takeuchi *et al.* teaches that every Cys within the protease domain is disulfide bonded. Takeuchi *et al.* teaches that Cys-830 is disulfide bonded to Cys-801, and not to Cys-191 as alleged by the Examiner. Takeuchi *et al.* teaches that the disulfide linkage between the protease domain and the pro-protease domain localizes the catalytic domain to the cell

surface. Thus, Takeuchi *et al.* does not teach or suggest a serine protease domain with a free Cys residue.

This further evidences that Takeuchi *et al.* does not teach or suggest an isolated single-chain protease domain. The protease domains of Takeuchi *et al.* are two-chain molecules; there is no free Cys as occurs in the instantly claimed single-chain protease domains.

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In view of the amendments and remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,

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